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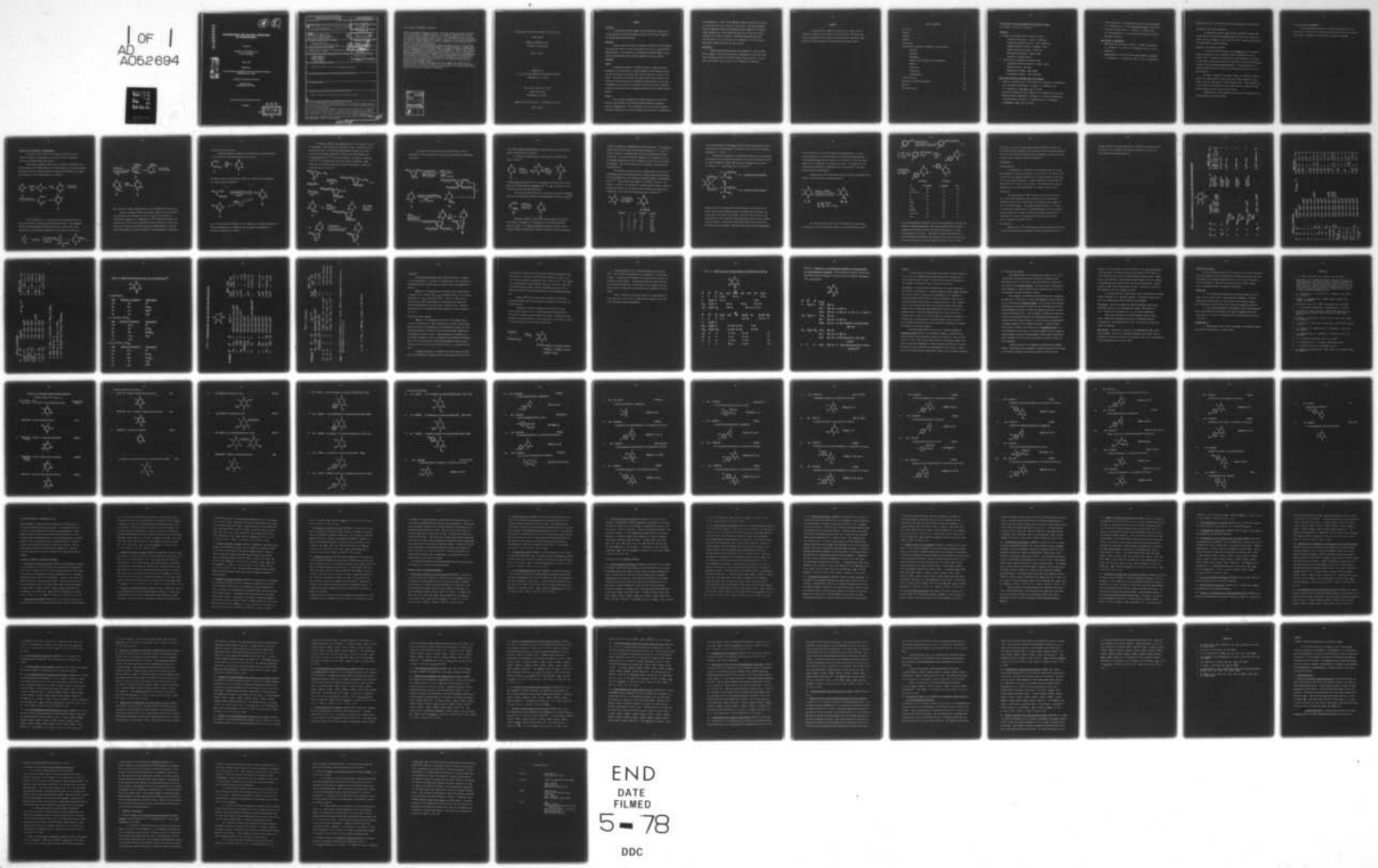

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OXAZINEDIONES AND RELATED COMPOUNDS AS ANTIMALARIALS

Final Report

STEPHEN S. WASHBURNE, Ph.D.
(Principal Investigator)

April 4, 1978

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D.C. 20314

CONTRACT NO. DAMD-17-74-C-4100

Temple University
PHILADELPHIA, PA 19122

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Objective- To develop new chemical agents for the treatment of malaria and related parasitic diseases by preparing samples of new organic compounds for testing against murine malaria.
Rationale- Because simple derivatives of compounds containing the oxazinedione ring system were known to exhibit inhibitory activity in E. coli and other microorganisms, it was hoped that a systematically prepared group of derivatives of oxazinediones would include compounds of activity against Plasmodia.

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BLOCK NUMBER 20 CONTINUED (Abstract)

Method- By standard techniques of organic synthesis, emphasizing those developed in this laboratory, to prepare samples of new compounds containing the oxazinedione ring system, and certain precursors to these new compounds. On the basis of results of testing against murine malaria, close chemically related derivatives of compounds showing modest activity would be prepared, with the goal of increasing the level of activity, hopefully to the point where one or more compounds would exhibit cures against murine malaria.

Results- Over sixty new compounds were prepared during the term of this contract, and submitted for screening in WRIR programs, principally against P. berghei (MM). Certain compounds were also screened by WRIR--designated laboratories for activity against schistosomiasis, leishmaniasis, and trypansomiasis. None of the submitted compounds exhibited statistically significant activity, nor were any cures shown. Two newly-developed, mutually compatible routes were developed for the preparation of oxazinediones (structure 1). Three papers were published in the open chemical literature as a result of synthetic methodology developed during the contract period, at least two more are in preparation, and one doctoral dissertation completed during the project period.

Conclusions- Substituted oxazinediones hold scant promise as a class of antimalarial agents. With the wide variety of substituents on the basic ring system developed during this contract showing limited promise, it is unlikely that further study of this class of compounds is warranted.

OXAZINEDIONES AND RELATED COMPOUNDS AS ANTIMALARIALS

FINAL REPORT

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SUMMARY

Objective -

To develop new chemical agents for the treatment of malaria and related parasitic diseases by preparing samples of new organic compounds for testing against murine malaria.

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Because simple derivatives of compounds containing the oxazinedione ring system were known to exhibit inhibitory activity in E. coli and other microorganisms, it was hoped that a systematically prepared group of derivatives of oxazinediones would include compounds of activity against Plasmodia.

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FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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Oxazinediones and Related Compounds as Antimalarial Agents

Final Report: 74 June 1 to 76 July 1

PERSONNEL -

A. Supported by Contract Funds in Part or in Full

Principal Investigator: Stephen S. Washburne

Postoctoral Research Associate: John H. MacMillan

Graduate Research Assistant: Kwanghee K. Park

Graduate Research Assistant: Ho Sull Lee

Undergraduate Assistant: Charles Pohan

Secretary: Christine Haines

B. Contributors not Supported by Contract Funds

Postdoctoral Research Associate: James D. Warren

Graduate Student: Lana King

Undergraduate Student: Mark Lenhart

Undergraduate Student: Alice Maragliano

PUBLICATIONS RESULTING FROM WORK UNDER THIS CONTRACT -

1. "Synthesis of Substituted 1,3-Oxazinediones by Reaction of Trimethylsilyl Azide with Maleic Anhydrides"; J. Warren, J. H. MacMillan, and S. S. Washburne, J. Org. Chem., 40, 743 (1975).

2. "Further Investigation of the Interaction of Trimethylsilyl Azide with Substituted Maleic Anhydrides. Synthesis of 4- and 5-Aryl Substituted 1,3(3H)-Oxazine-2,6-diones"; J. H. MacMillan and S. S. Washburne, J. Heterocycl. Chem., 12, 1215 (1975).

3. "Ethyl Oxaorotate -- A New Synthetic Route to 1,3-Oxazinediones"; S. S. Washburne and K. K. Park, Tetrahedron Letters, 243 (1976).

4. "Syntheses of Heterocycles. I. Triazoles. II. Orotic Acids. III. Oxazinediones"; K. K. Park, Doctoral Dissertation, Temple University, May 1976.

PUBLICATIONS IN PREPARATION --

1. "Hydrolysis of 1,3-Oxazine-2,6-diones: Products and Kinetics" S. S. Washburne, K. K. Park, J. H. MacMillan, and H. S. Lee, in preparation.

2. "Regiochemistry of Attack of Silyl Azides on Maleic Anhydrides" S. S. Washburne, J. H. MacMillan, and H. S. Lee, in preparation.

REPORT OF ACTIVITY "Oxazinediones and Related Compounds as Antimalarials"

Statement of the Problem--OBJECTIVE

To develop new chemical agents for the treatment of malaria and related parasitic diseases by preparing samples of new, novel organic compounds; these new compounds to be tested for activity against murine malaria in standard WRAIR screening programs.

Approach to the Problem--RATIONALE

It is not considered axiomatic that Plasmodia do not incorporate exogenous pyrimidines, but do depend on purines supplied by the host (Pinder, 1973). Pyrimidine anti-metabolites can be expected to interfere at two stages in plasmodial nucleic acid sysnthesis; blockage of thymidylate synthetase and/or dihydroorotatedehydrogenase. Several pyrimidine anti-metabolites have been shown capable of interfering with parasitic nucleic acid synthesis.

The parent compound of the target series, 1,3-oxazine-2,6-dione is known to be active against various tumor cell cultures, against E. coli (Skoda, 1973-77), and against S. faecium (Bobek, 1975). These reports led to the selection of oxazinediones as a target series of potential utility against parasitic disease, specifically malaria.

Fuller details of the rationale may be found in the proposals submitted originally for this contract.

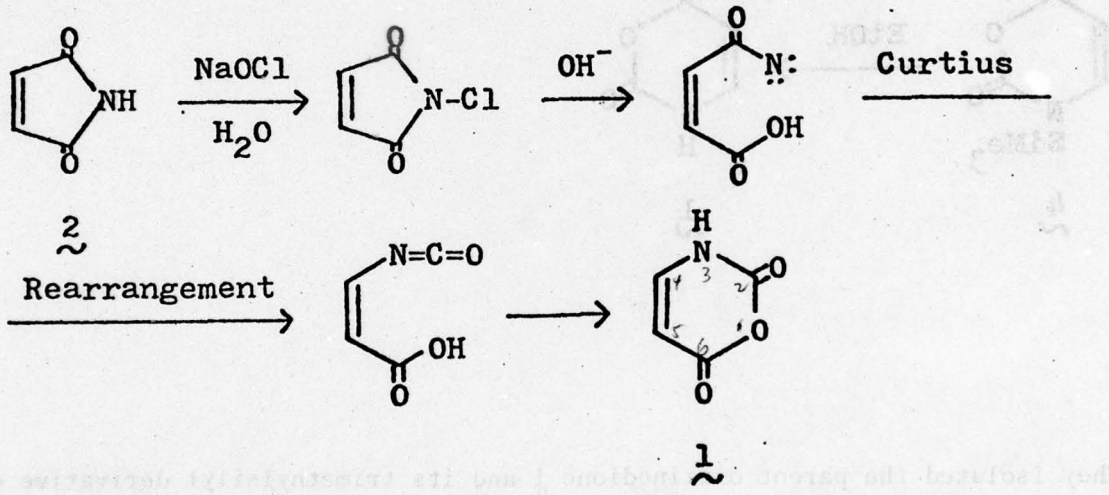
The Target Series--BACKGROUND

The following pages constitute a review of the chemistry of the target series. Much of the work described was completed during the contract period and is a fundamental contribution to scientific knowledge.

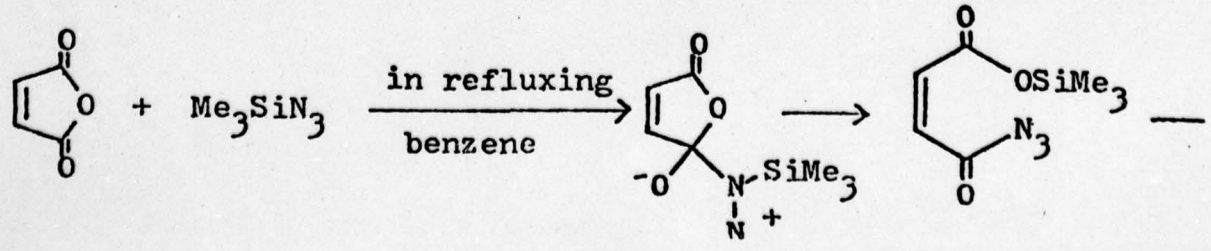
ASPECTS OF THE CHEMISTRY OF OXAZINEDIONES: -

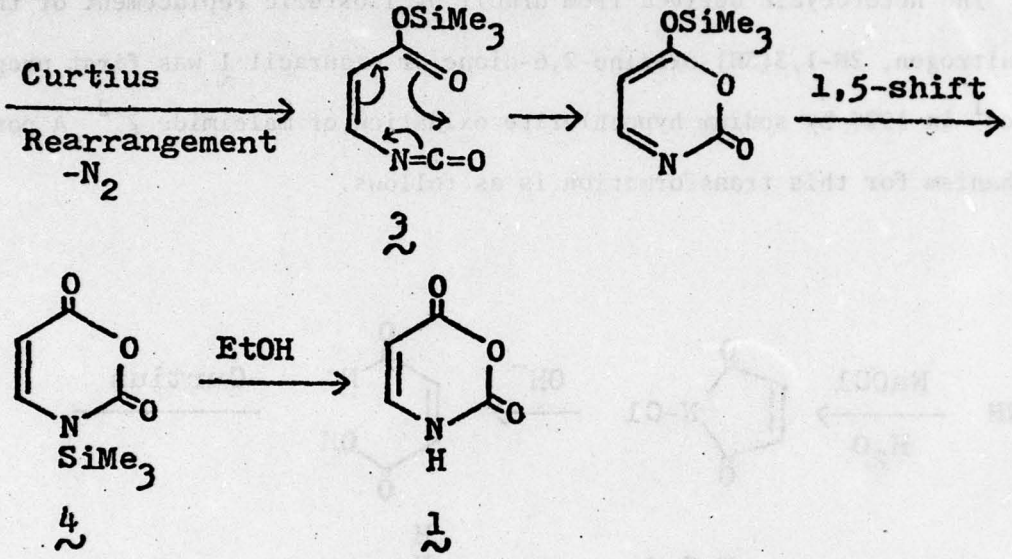
The object of this section is to present an overview of the synthetic chemistry, spectroscopy, and reactivity of the 1,3-oxazine--2,6-diones developed through this contract.

The heterocycle derived from uracil by isosteric replacement of the imidic nitrogen, 2H-1,3(3H)-oxazine-2,6-dione or oxauracil 1 was first prepared by Rinkes[1] in 1927 by sodium hypochlorite oxidation of maleimide 2.[2] A possible mechanism for this transformation is as follows.



In 1972 Washburne et. al. reported an alternate preparation of 1 by reaction of maleic anhydride with trimethylsilylazide.[3] They suggested that the mechanism probably involves electrocyclic ring closure of isocyanatoacrylate 3. followed by sigmatropic 1,5-trimethylsilyl migration.



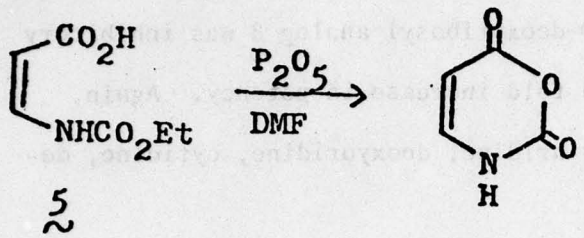


They isolated the parent oxazinedione 1 and its trimethylsilyl derivative 4.

Shortly thereafter, Škoda and coworkers reported the growth inhibitory properties of oxazinedione 1 versus E. coli.[4] They reported the inhibition was complete at a concentration of 10^{-4} M and the inhibition was reversed with 10^{-4} M uridine, cytidine and partly with uracil, but not with orotic acid, cytosine, purine bases and purine ribonucleosides. They concluded oxazinedione 1 is a novel inhibitor of the biosynthesis of pyrimidine

precursors of nucleic acids.

Bobek and coworkers reported a third synthesis of 1 which involves cyclization of an alkoxycarbonylaminoacrylic acid 5.[5]



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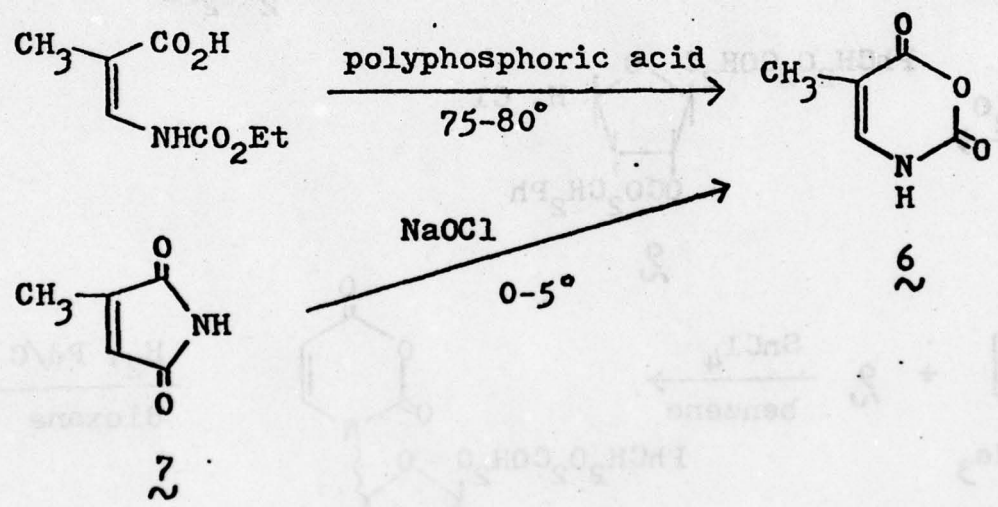
1

By similar methods, they prepared 5-methyl-2H-1,3(3H)-oxazine-2,6-dione 6, the oxygen isoster of thymine.[5]

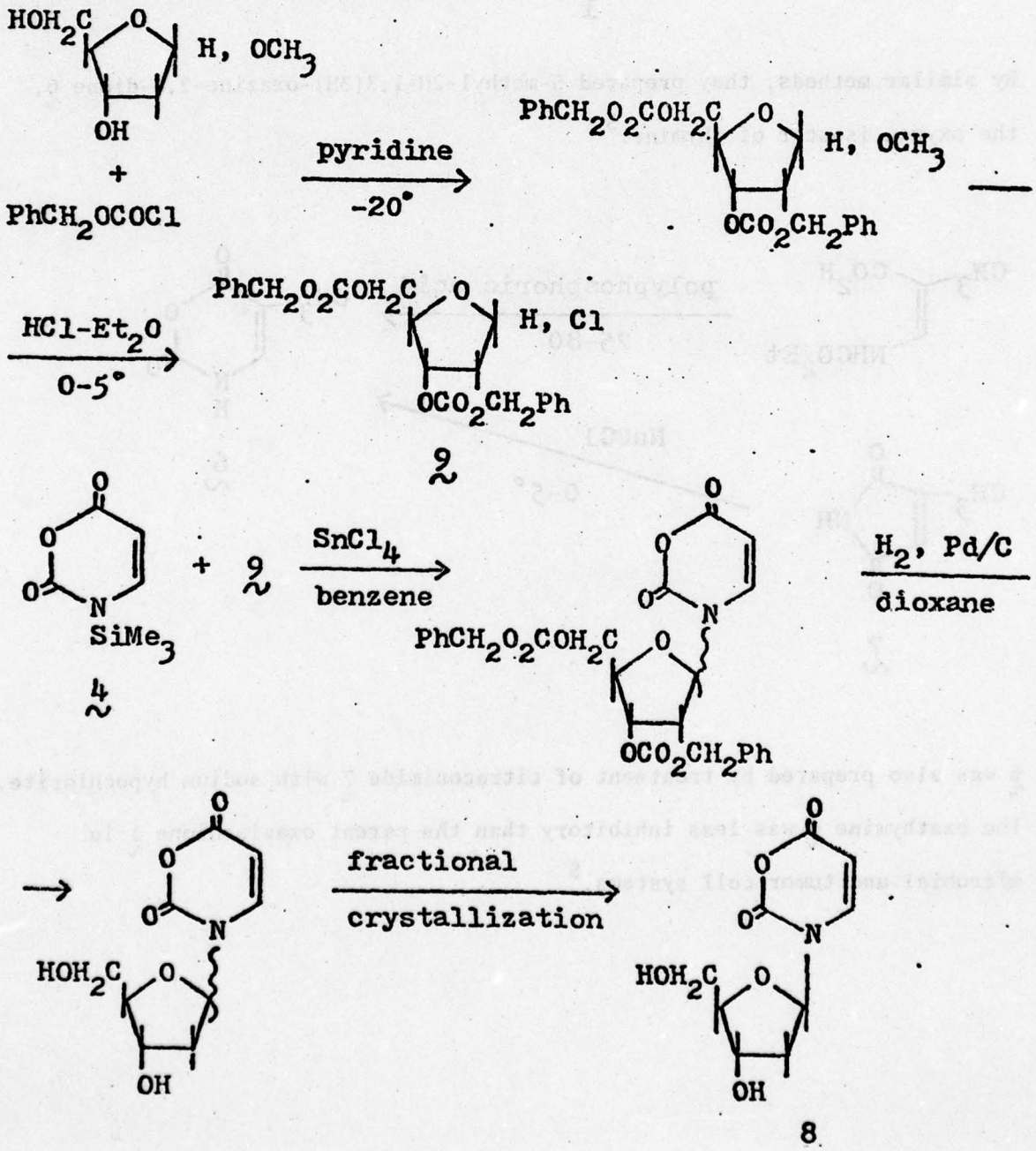


7

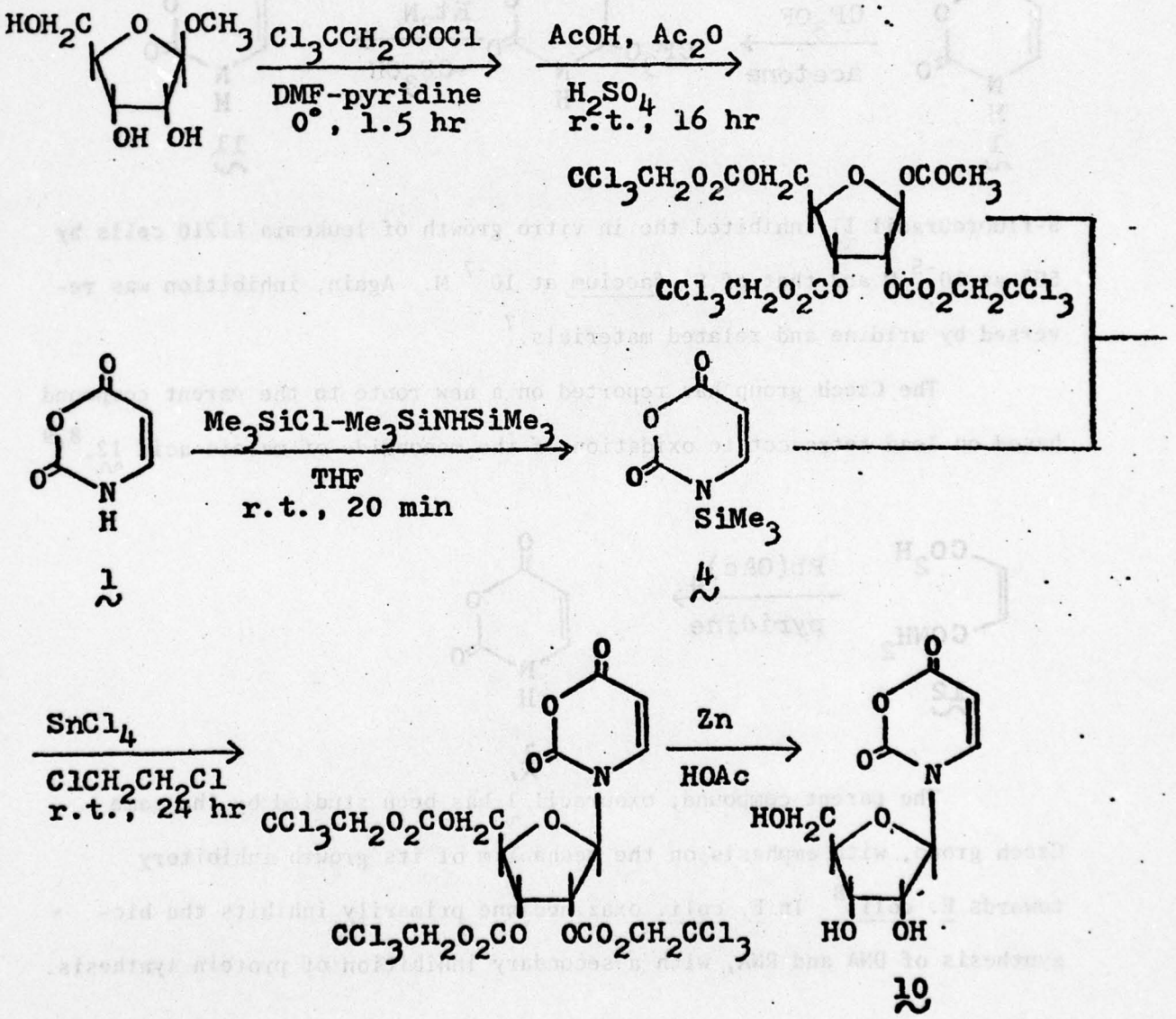
6

6 was also prepared by treatment of citraconimide 7 with sodium hypochlorite.[2] The oxathymine 6 was less inhibitory than the parent oxazinedione 1 in microbial and tumor cell systems.[5]

Although 1 inhibited the *in vivo* growth of L-1210 leukemia in mice, the therapeutic index obtained was relatively narrow. To possibly achieve greater selectivity, 3-(2-deoxy-β-D-erythro-pentofuranosyl)-2H-1,3(3H)-oxazine-2,6-dione 8 was synthesized.[5] While 1 gave 50% inhibition of *S. faecium* growth at 10^{-5} M, the β-D-deoxyribosyl analog 8 was inhibitory at 5×10^{-8} M, an approximately 1000 fold increase in potency. Again, inhibition was partially reversed by uridine, deoxyuridine, cytidine, deoxycytidine and by thymidine.[5]

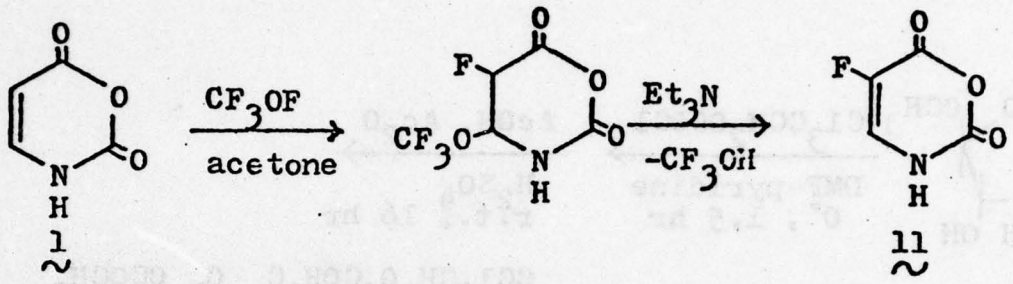


The synthesis of the ribonucleoside of oxazinedione, 3-(β-D-ribofuranosyl)-2H-1,3(3H)-oxazine-2,6-dione 10 was reported by Heidelberger and Chwang.[6]



The N-riboside 10 had approximately the same activity as 1 in inhibiting growth of L5718Y cells in culture.[6]

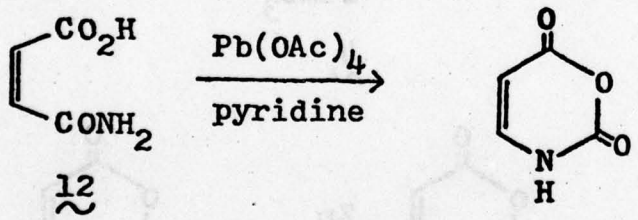
5-fluoro-2H-1,3(3H)-oxazine-2,6-dione 11 was synthesized by Bobek and Bloch.[7]



1 11

5-fluorouracil 11 inhibited the in vitro growth of leukemia L1210 cells by 50% at 10^{-5} M and that of S. faecium at 10^{-7} M. Again, inhibition was reversed by uridine and related materials.[7]

The Czech group has reported on a new route to the parent compound based on lead tetraacetate oxidation of the monoamide of maleic acid 12.[8,9]

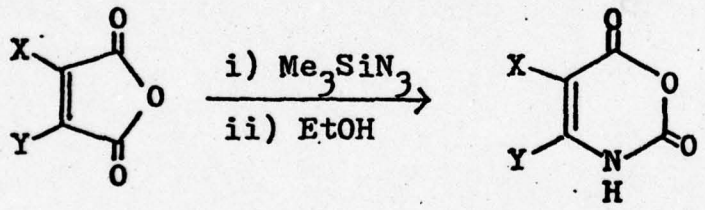


12 1

The parent compound, oxauracil 1 has been studied by the same Czech group, with emphasis on the mechanism of its growth-inhibitory towards E. coli.[8] In E. coli. oxazinedione primarily inhibits the biosynthesis of DNA and RNA, with a secondary inhibition of protein synthesis.

It does not inhibit the dihydroorotate-orotate conversion. The inhibitory effect can be relieved only with preformed pyrimidines, e.g., uracil or uridine; aspartate, ureidosuccinate, orotate and dihydroorotate being ineffective. It was also shown that hydrolysis of 3-oxauracil 1 at 37° in an aqueous medium gives rise to formylacetic acid as the sole product of hydrolysis. The rate of hydrolysis of 3-oxauracil 1 increases with rising pH-value.[8]

The reported biological acitvity, and potential utility of the oxazinedione ring system against parasitic disease, particularly malaria, led this laboratory to detail improved synthetic pathways to these heterocycles.[10,11] They reported the synthesis of 4-bromo-, 4-chloro-, 4,5-dichloro-, 4-fluoro-, and 4-methyl-2H-1,3(3H)-oxazine-2,6-dione, as well as an improved synthesis of 2H-1,3(3H)-oxazine-2,6-dione 1, by reaction of trimethylsilyl azide with the corresponding maleic anhydride.[10]

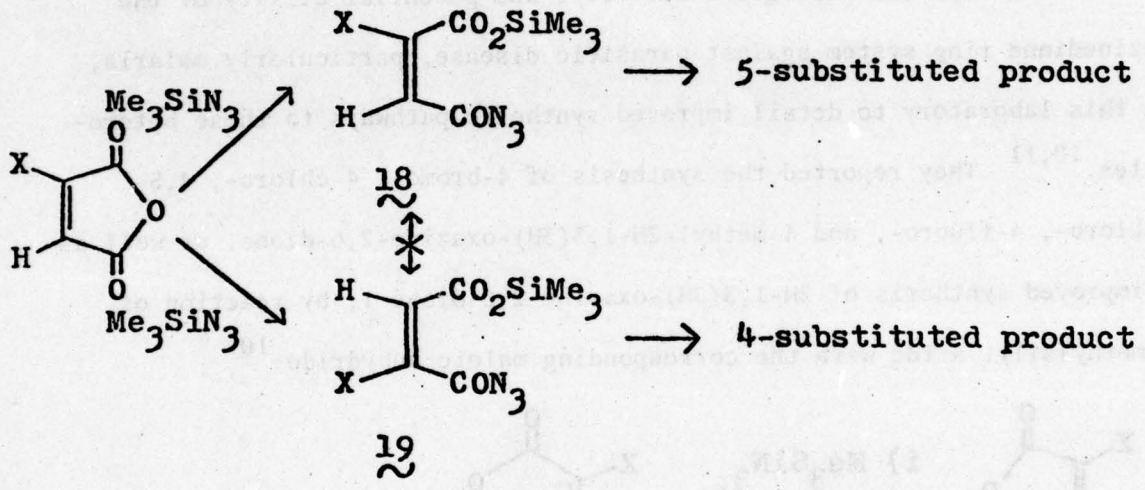


1, 13-17

Compound	X	Y	Yield(%)	Solvent
1	H	H	69	$CHCl_3$
13	H	CH_3	33	CHCl
14	H	Br	30	neat
15	H	Cl	57	neat
16	H	Cl	38	$CHCl_3$
17	H	F	10	$CHCl_3$

Since the oxazinedione ring undergoes facile thermal decarboxylative polymerization to yield polyamides,[12] the reaction was carried out at moderate temperature, i.e. <100°.

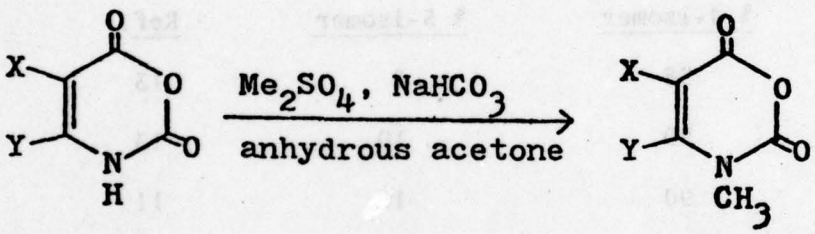
Preferential formation of 4-substituted products over 5-substituted oxazinediones was explained by assuming that the initial nucleophilic attack of azide at anhydride carbonyl determines the regiochemistry of product oxazinedione; i.e., 18 and 19 do not interconvert.[10]



Obviously, steric interference by the substituent is not product determining since the more hindered carbonyl is preferentially attacked, even in the case of the bulky bromine group. Electronic factors must be rate determining for these systems and the azide should attack the most electropositive carbonyl. For the halo substituents simple inductive electron withdrawal from the proximate carbonyl by the electronegative halogen makes

makes the carbonyl α to the substituent more electropositive. For the methyl substituent a conjugative interaction between the substituent and the double bond feeds electron density to the carbonyl β to the substituent making it less susceptible to nucleophillic attack. The nonreactivity of dimethylmaleic anhydride is consistent such an interaction,[10] since both carbonyls would be deactivated.

N-Methylation of the oxazinedione ring was readily accomplished with dimethyl sulfate buffered by sodium bicarbonate.

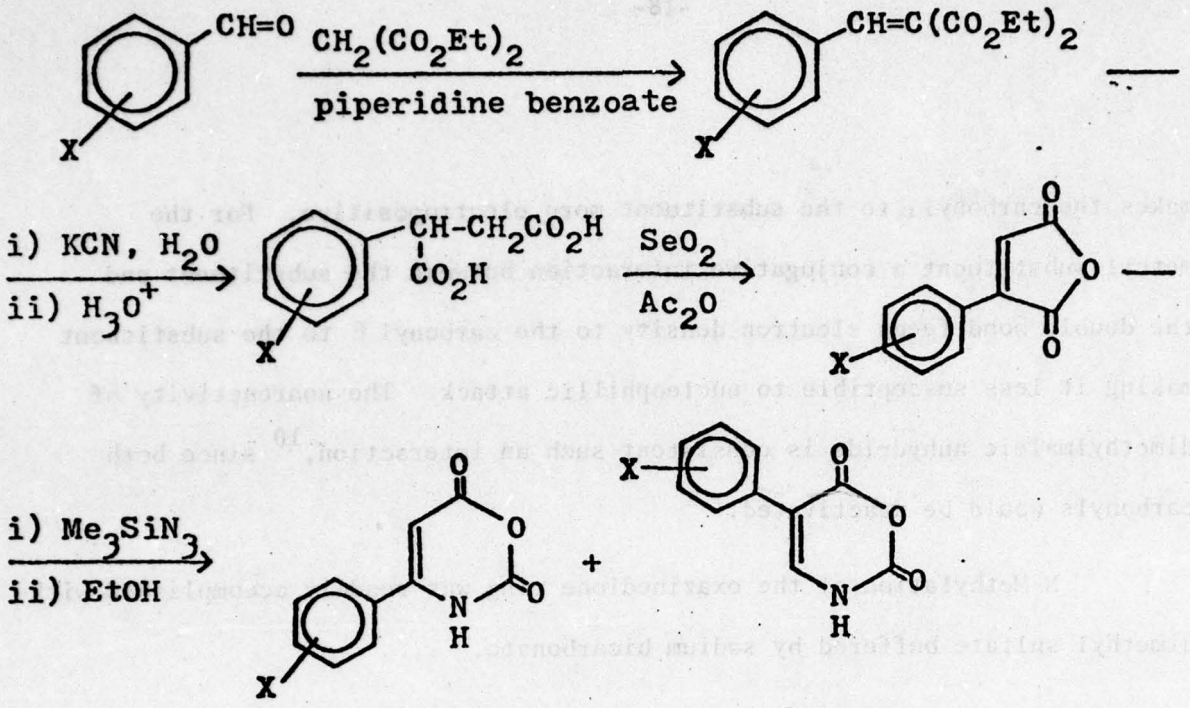


1, 13 — 20, 21

1, 20; X=Y=H

13, 21; X=H, Y=CH_3

Aryl substituted 2H-1,3(3H)-oxazine-2,6-diones were synthesized by reacting aryl substituted maleic anhydrides with trimethylsilyl azide.[11]



4-isomer 5-isomer

X	% 4-isomer	% 5-isomer	Ref.
p-CH_3	98	2	13
3-Cl-4-CH_3	90	10	13
H	90	10	11
p-F	80	20	11
p-Cl	70	30	11
p-Br	70	30	13
p-OCH_3	70	30	11
3,4-di-Cl	60	40	11
o-Cl	55	45	11

In all cases mixtures of isomeric 4- and 5-aryl-oxazinediones were obtained with the 4-isomer predominating. The isomeric mixture could be readily separated by fractional crystallization from ethyl acetate or column chromatography on silica gel. The yield of 5-isomer was greatest for o-chlorophenylmaleic anhydride, and substantial for other arylmaleic anhydrides, indicating the increased importance of steric effects in these

reactions, in contrast to previously reported syntheses of methyl- and halo-substituted oxazinediones, where electronic factors appeared dominant.[11] These aryl-substituted oxazinediones were N-methylated in good yields with dimethyl sulfate buffered by sodium bicarbonate.[11]

SPECTROSCOPY

Infrared Spectra

A characteristic ir spectrum of an oxazinedione has two strong sharp carbonyl stretching absorptions at 1815-1780 cm^{-1} (C_6 carbonyl) and at 1755-1710 (C_2 carbonyl) together with a strong absorption at 1670-1602 cm^{-1} (C_4-C_5 alkene stretching). A broad absorption in the 3400-3100 cm^{-1} range for the N-H stretch is also visible. The characteristic infrared absorptions in the range 1850-1600 cm^{-1} are shown in Table 1 and 3.

NMR Spectra

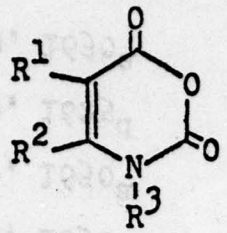
The characteristic ^1H NMR resonances for the C_5 proton are in the 5.28-5.95 ppm region while the C_4 proton (β to the C_6 carbonyl) resonates in the 8.35-7.46 ppm region. The N-methyl protons appear in the 3.10-3.44 ppm region. The C_5-methyl protons resonate at 1.83 ppm in 5-methyl-2H-1,3(3H)-oxazine-2,6-dione(oxathymine) while the C_4-methyl protons resonate at 2.08 ppm in 4-methyl-2H-1,3(3H)-oxazinedione-2,6-dione and at 2.4 ppm in 3,4-dimethyl-2H-1,3(3H)-oxazine-2,6-dione. Data are summarized in Table 1 and 3.

Mass Spectra

Washburne et. al.[10] reported mass spectroscopic data for N-protio and N-methyl-substituted oxazinediones, which are summarized in Table 2.

Dominant features of the mass spectra are a moderate to strong molecular ion together with a prominant M-44 peak corresponding to extrusion of carbon dioxide from the molecular ion.

Table 1. Characteristic ir, uv and nmr data for Oxazinediones



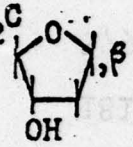
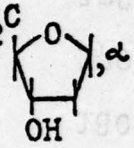
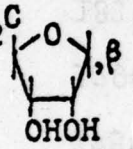
R^1	R^2	R^3	ir(cm^{-1})	uv, λ_{max}, nm	nmr(δ, ppm) C_5-H	C_4-H	N-H	Ref.
H	H	H	1780, 1700[a] 1790, 1710, 1635[a] 1785, 1715, 1634[b]	264(H_2O)[e] log∈, 3.90	5.59[f] 5.56[f] 5.48[f]	7.75 7.52 7.46	10 10.75 11.40	3 10 8
CH_3	H	H		271(EtOH) ∈6,500	(1.83)7.57[f]			5
H	H			269(EtOH) ∈7,150	5.68[f]	8.13		5
H	H			268(EtOH) ∈7,100	5.68[g]	8.02		5
H	H		1785, 1720[c]	265(H_2O) log∈, 3.79	5.92[f]	8.35		6
H	CH_3	H	1790, 1710, 1640[a]		5.38[f]	(2.08)11.5		10

Table 1. continued

R^1	R^2	R^3	ir(cm^{-1})	uv, λ_{max}, nm	nmr(δ, ppm) C_5-H	C_4-H	N-H	Ref.
H	Br	H	1780, 1602[a]		5.87[f]	---	11.0	10
H	Cl	H	1790, 1725, 1615[a]		5.84[g]	---	11.0	10
Cl	Cl	H	1815, 1755, 1605[a]		---	---	9.9[f]	10
H	F	H	1800, 1750, 1670[a]		5.36[g]	---	8.5	10
H	H	CH_3	1790, 1745, 1715, 1640[d]		5.65[f]	7.75	(3.2)	10
H	CH_3	CH_3	1780, 1720, 1630[d]		5.60[f]	(2.4)	(3.4)	10
H	Ph	H	1810, 1710, 1630[a]		5.65[f]	---	11.0	11
H	Ph	CH_3	1790, 1720, 1630[d]		5.28[f]	---	(3.10)	11
H	p-anisyl	H	1790, 1720, 1630, 1605[d]		5.95[f]	---	11.6	11
H	p-anisyl	CH_3	1780, 1720, 1620, 1610[d]		5.55[f]	---	(3.10)	11
p-anisyl	H	CH_3	1790, 1730, 1640, 1610[d]		---	8.0[f]	(3.4)	11
H	o-Cl-Ph	H	1800, 1730, 1640[d]		5.80[f]	---	9.2	11
o-Cl-Ph	H	H	1800, 1730, 1640[d]		i	---	9.2	11
H	p-Cl-Ph	H	1790, 1710, 1630[a]		5.6[f]	---	11.0	11
p-Cl-Ph	H	H	1780, 1710, 1630[a]		---	7.65[f]	10.8	11
H	p-Cl-Ph	CH_3	1790, 1730, 1625[d]		5.56[h]	---	(3.33)	11
H	3,4-di-Cl-Ph	H	1790, 1710, 1630[a]		5.75[f]	---	11.0	11

Table 1. continued

R^1	R^2	R^3	ir(cm^{-1})	uv, λ_{max}, nm	nmr(δ, ppm) C_5-H	C_4-H	N-H	Ref.
3,4-di-Cl-Ph	H	H	1780, 1720, 1630[a]		---	7.56[f]	10.0	11
H	3,4-di-Cl-Ph	CH_3	1790, 1730, 1630[d]		5.80[g]	---	(3.2)	11
3,4-di-Cl-Ph	H	CH_3	1790, 1730, 1640[d]		---	8.1[g]	(3.44)	11
H	p-F-Ph	H	1780, 1725, 1705, 1625[a]		5.84[g]	---	10.0	11
H	p-F-Ph	CH_3	1785, 1720, 1625[d]		5.68[f]	---	(3.28)	11
p-F-Ph	H	CH_3	1790, 1730, 1640[d]		---	8.05[f]	(3.40)	11

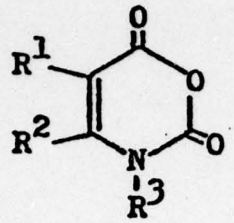
a: mull, b: KBr, c: fluorolube, d: $CHCl_3$ or $CDCl_3$

e: taken from ref. 4)

f: DMSO-d_6, g: acetone-d_6, h: $CDCl_3$

i: C_5-H overlapped with aromatic hydrogens.

Table 2. Mass Spectroscopic data for Oxaziniediones[10]



i) $R^1=R^2=R^3=H$

m/e	relative intensity	assignment
113	58	M^+
69	100	$M-CO_2$
43	52	$HNCO^+$
44	52	CO_2^+

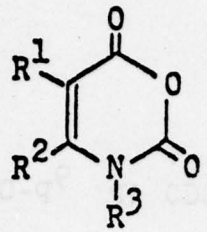
ii) $R^1=R^3=H$, $R^2=CH_3$

m/e	relative intensity	assignment
127	57.2	M^+
83	46	$M-CO_2$
68	46	$M-HNCO_2$
44	41.3	CO_2^+
42	100	

iii) $R^1=R^2=H$, $R^3=CH_3$

m/e	relative intensity	assignment
127	100	M^+
83	132	$M-CO_2$
55	109	$M-C_2O_3$
44	70	CO_2^+
42	190	CON^+

Table 3. Characteristic ir and nmr data for Oxaziniediones



Compound	R^1	R^2	R^3	ir(cm^{-1})	nmr(δ, ppm) C_5-H	C_4-H	N-H
	CH_3	CH_3	H	1780,1760,1730,1715,1650[a]	(1.83)[c]	(2.08)	11.14
	H	CO_2Et	H	1805,1745,1730,1710,1650[a]	6.31[d]	---	8.5
	CH_3	CO_2Et	H	1800,1740,1720,1640[a]	(2.28)[d]	---	8.63
	$-CH_2CH_2CH_2-$		H	1775,1740,1645[a]	---	---	11.60[c]
	H	$ClCH_2$	H	1785,1725,1640[a]	5.74[c]	(4.34)	11.78
	Cl	CH_3	H	1815,1785,1725,1705,1635,1620[a] 1790,1725,1635[b]	---	(2.23)[c]	11.92
	H	CH_2F	H	1790,1740,1655[a]	5.62[3]	(5.11)	11.64
	H	CF_3	H	1815,1760,1680[a]	5.98[c]	---	9.6
	CH_3	CH_3	CH_3	1770,1720,1630[a]	(1.95)[d]	(2.23)	(3.39)
	H	CO_2Et	CH_3	1785,1730,1630[a]	6.03[d]	---	(3.48)

Table 3. Continued

Compound	R^1	R^2	R^3	ir(cm^{-1})	nmr(δ, ppm) C_5-H	C_4-H	N-H
	CH_3	CO_2Et	CH_3	1785,1740,1725,1650[a]	(1.90)[d]	---	(3.29)
	$-CH_2CH_2CH_2-$		CH_3	1775,1730,1635[a]	---	---	(3.34)
ref. 15	Cl	H	H	1739sh, 1779, 1730, 1711, 1634[e]	---	7.82[c]	not observed
ref. 15	Br	H	H	1788, 1743, 1727, 1644, 1617[e]	---	7.97[c]	not observed
ref. 15	I	H	H	1790sh, 1780, 1724, 1711, 1621[e]	---	7.82[c]	not observed

Note: a nmr resonance in brackets, e. g. (1.90), refers to absorption of protons on a group bonded to C_5 or C_4 or N

a: mull b: $CDCl_3$ c: DMSO-d_6 d: $CDCl_3$ e: KBr pellet

UV Spectra

Ultraviolet measurements were reported for parent-, 5-methyl-, 3-(β-D-ribofuranosyl)-, 3-(2-deoxy-α-D-ribofuranosyl)- and 3-(2-deoxy-β-D-ribofuranosyl)-2H-1,3(3H)-oxazine-2,6-diones, which are summarized in Table 1.

Škoda et. al.[8] reported the uv spectrum of 2H-1,3(3H)-oxazine-2,6-dione in 0.1 m HCl(pH 1.20): λ_{max} 264 nm($\log^{\varepsilon}$ 3.949); in 0.1 M borate buffer(pH 11.2): λ_{max} 296 nm($\log^{\varepsilon}$ 4.046). They also examined the uv spectra of the parent oxazinedione at various pH values, which made possible determination of its pKa value, 7.78±0.02 at 25°. Similarly the pKa value of 4,5-dimethyl-2H-1,3(3H)-oxazine-2,6-dione was found to be 8.67.[14]

Hydrolysis of Oxazinediones

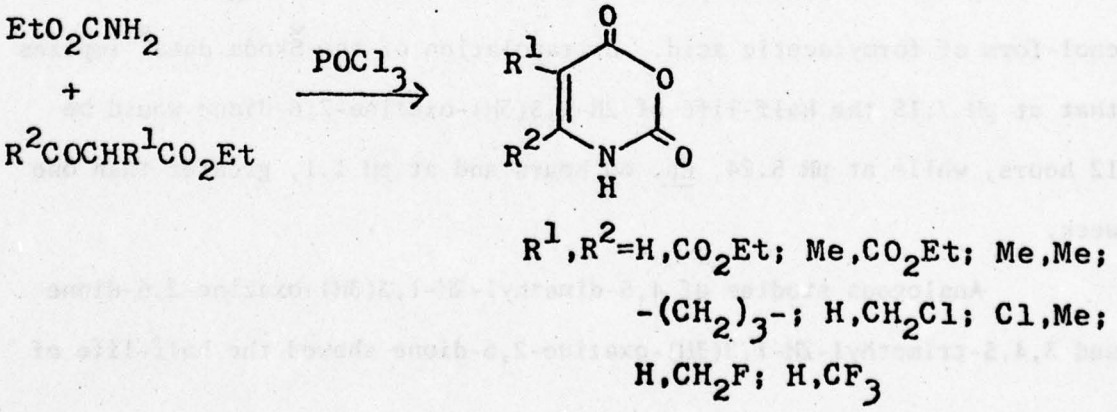
Škoda et. al.[8] reported the stability of 2H-1,3(3H)-oxazine-2,6-dione at various pH's. When recording the uv spectra of 2H-1,3(3H)-oxazine-2,6-dione, the absorbance at 296 nm decreased steeply and a new abosorption maximum at 258 nm was observed which maximum disappeared on acidifying the solution to pH 4. The new maximum was assigned to the enol-form of formylacetic acid. Extrapolation of the Škoda data[8] implies that at pH 7.15 the half-life of 2H-1,3(3H)-oxazine-2,6-dione would be 12 hours, while at pH 5.24, ca. 60 hours and at pH 1.1, greater than one week.

Analogous studies of 4,5-dimethyl-2H-1,3(3H)-oxazine-2,6-dione and 3,4,5-trimethyl-2H-1,3(3H)-oxazine-2,6-dione showed the half-life of

the former is 20 hours at pH 9.18, 45 hours at pH 8.25 and greater than 18 days at pH 7, while the half-life of the latter is 2.5 hours at pH 9.18, 23 hours at pH 8.25 and greater than 17 days at pH7.[14] This implies that 4,5-dimethyl-2H-1,3(3H)-oxazine-2,6-dione is much more stable than 2H-1,3(3H)-oxazine-2,6-dione, and the N-methylated derivative of the former oxazinedione is much less stable than the corresponding N-protio one.

Recent work[15] by the Czech group has further clarified the hydrolysis pathways open to substituted oxazinediones of the 5-halo and 5-methyl calsses.

Condensation of ethyl carbamate with a variety of β-keto-esters affords modest yields of 4-substituted and 4,5-disubstituted 1,3-oxazine-2,6-diones inaccessible by other routes. Among these oxygen isosteres of uracil derivatives, ethyl oxaorotate is of principal interest as a physiologically active analog of orotic acid.

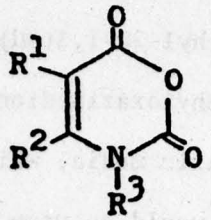


R^1, R^2=H,CO_2Et; Me,CO_2Et; Me,Me; $-(CH_2)_3-$; H,CH_2Cl; Cl,Me; H,CH_2F; H,CF_3

These compounds as well could be N-methylated in excellent yield. The hydrolytic decomposition of 4-carboethoxy-, 4-carboethoxy-5-methyl-, 4-carboethoxy-3,5-dimethyl-2H-1,3(3H)-oxazine-2,6-dione was studied in various buffers. N-methyloxazinedione was hydrolyzed much faster than the N-protio one in basic media, which suggested that the initial attack of hydroxide anion would be upon C_6-carbonyl of oxazinediones.

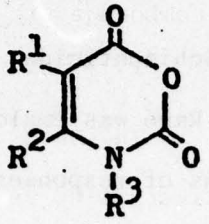
Table 4 summarizes the kinetic stability of oxazinediones at various pH levels, while Table 5 illustrates the changes in the ultraviolet spectrum as hydrolysis proceeds.

Table 4. Half-lives of oxaziniediones at various pH values



R^1	R^2	R^3	pH 1.1	1.6	5.24	5.8	6.0	7.0	7.15	7.56	9.2	10	10.38	11	11.36	Ref
H	H	H	>week		60 hr				12 hr	9 hr			5.5 hr		3.5 hr	8
H	CO_2Et	H	72 hr					6 hr								
CH_3	CO_2Et	H		61 hr		29 hr		16.5 hr			4.7 hr	2.9 hr		1 hr		
CH_3	CO_2Et	CH_3		108 hr			28.5 hr	3.4 hr			<5 min	<5 min		<5 min		
CH_3	H	H									4.2 hr		4.5 hr			15
Br	H	H									8 hr		2.7 hr			15
Cl	H	H									4.0 hr		1.9 hr			15
I	H	H									>8 hr		5.3 hr			15

Table 5. Changes in uv absorption maxima as decomposition of oxaziniediones proceeds. The absorption maxima shown last decreases proportionally in each case as further decomposition progresses.



R^1	R^2	R^3		λ_{max}
H	CO_2Et	H	acid	288 nm
			pH 7	328 nm → 308 nm
			base	328 nm → 308 nm → 274 nm → 288 nm
CH_3	CO_2Et	H	acid	291 nm
			pH 7	305 nm → 290 nm
			base	313 nm → 302 nm(after acidification, 287 nm)
CH_3	CO_2Et	CH_3	acid	281 nm
			pH 7	281 nm → 284 nm
			base	281 nm → 286 nm(within less than 5 min)
H	H	H	base	296 nm → 258 nm(disappears on acidification)[8]

RESULTS

The main fruit of research under this contract was the preparation of over sixty new compounds, which were submitted for screening in the murine malaria program of WRAIR. Structures and synthetic procedures to the compounds are detailed in later sections of this report.

Test Method - The standard Blood Schizonticidal Test (Mouse) developed at the University of Miami by Dr. L. Rane was employed on the samples submitted. This system is based on comparisons of responses to test compounds by Plasmodium berghei KBG 173 malaria in mice as expressed in mean survival times and the mean survival times of untreated controls. Thus, compounds noted as active produce increases in the survival times of the treated animals that are significant when compared with the survival times of untreated controls. Since an established disease is less sensitive to treatment than a disease in the early stages of development, treatment is withheld until the parasitemia is relatively high in order to insure a more reliable assay of activity and the selection of appropriate compounds for intensive pre-clinical studies.

Utilizing young ICR/HA Swiss mice and a standard inoculum of Plasmodium berghei KBG 173, it is possible to produce a uniform disease fatal to 100% of untreated animals within 6 to 8 days with a mean survival time of 6.2 days. Test animals weigh from 18 to 22 grams but weight variations in any given experimental or control group are confined to 2-3 grams. All animals in any given test are approximately of the same age. Animals on test are housed in metal-topped plastic cages, given a standard laboratory

diet and water ad libitum.

Test animals receive an intraperitoneal injection of 0.5 ml of 1:100 dilution of heparinized heart's blood with a minimum of 90% parasitized cells (4×10^7 cells), drawn from donor mice infected one week earlier with Plasmodium berghei. The donor strain is maintained by weekly passages in separate groups of mice inoculated with a 0.5 ml of 1:500 dilution of heparinized heart's blood.

Test compounds are administered after dissolution or suspension in peanut oil. A single dose is given subcutaneously 72 hours after the mice are infected with Plasmodium berghei. At this time a 10-15 percent parasitemia has developed; the disease is well established but has not produced sufficient debility to alter the response of the host to toxic effects of the drug on test. Since treatment is withheld for three days to permit the infection to become well established and death occurs in untreated controls within 6-8 days, it is felt that this system presents a candidate compound with the maximum challenge. In order to check factors such as changes in the infectivity of Plasmodium berghei or in the susceptibility of the host or to detect technical errors, a group of infected animals treated with pyrimethamine at does levels producing definite increases in survival time is included as a positive control in every experiment.

In each experiment test compounds are administered in graded dosages. With highly active compounds, increases in dose levels are usually followed by increases in the survival time of the treated mice.

However, if an active drug is toxic for the host, its toxicity may become a limiting factor; continued increases in dose levels also increase the toxic effects and may result in the diminution of survival times. Deaths prior to the sixth day, when untreated controls begin to die, are regarded as non-parasitic and become the basis for toxicity evaluations. Treated animals are kept under observation for 60 days. Survivors at the end of this period of time are considered as cured.

An increase of 100% in mean survival time is considered the minimum effective response for a candidate compound. In calculating mean survival time, toxic deaths and 60 day survivors are not included.

Test Results - None of the submitted compounds exhibited statistically significant activity in the indicated test system. The highest T-C (Test - Control) level observed was, 0.9, well below significance.

Other tests performed on selected compounds from the target series included the leishmaniasis (University of Georgia, Hanson), schistosomiasis, and tyrpansomiasis screens. No activity in these test systems was observed.

Other Results - Chemically, two routes to the oxazinedione target series were developed. These routes, mutually compatible in that they lead to differently substituted derivatives of the parent ring system, are discussed in the background section of this report.

DISCUSSION OF RESULTS

While the organic chemistry of the target series was well developed, and several groups of interest attached to the target ring system, the testing results were uniformly unpromising; that is, no activity in parasitic test system observed. The lack of any activity over a widely ranging series of compounds leads to an important conclusion:

CONCLUSIONS

1. The oxazinedione ring system, for whatever reason (as yet unknown) is not an active part of any antimalarial compounds. Although a combination of substituents may lead to some activity against malaria, that combination is not present in any of the sixty compounds submitted.

2. The rationale advanced in the proposal; that 1,3-oxazinediones being antimetabolites, particularly in E. coli, S. faecium, and various tumor cell lines, would be of potential utility against parasitic disease, is not true.

RECOMMENDATION

1. That further studies for the development of antimalarial agents not include oxazinediones as a target series.

REFERENCES

1. I. J. Rinkes, Recl. Trav. Chim. Pays-Bas, 46, 268 (1927).

2. Washburne et. al.[10] reported the Rinkes hypochlorite oxidation of either maleimide or citraconimide did not yield the corresponding oxazinedione even after several attempts in which the pH was carefully controlled. Instead, N-chloro-maleimide was isolated with either sodium or calcium hypochlorite in aqueous acetic acid. The N-chloro-maleimide did rearrange to 2H-1,3(3H)-oxazine-2,6-dione in aqueous sodium bicarbonate solution.

3. S. S. Washburne, W. R. Peterson and D. A. Berman, J. Org. Chem., 37, 1738(1972).

4. J. Škoda, Z. Felegelova and J. Farkaš, Biochem. Biophys. Res. Commun., 50, 80(1973).

5. M. Bobek, A. Bloch and S. Kuhar, Tetrahedron Lett., 3493(1973).

6. T. L. Chwang and C. Heidelberger, Tetrahedron Lett., 95(1974).

7. M. Bobek and A. Bloch, Abstracts, 168th National Meeting of the American Chemical Society, Atalntic City, N.J., Sept. 1974, Med. #65.

8. J. Škoda, I. Votruba and J. Farkaš, Collect. Czech. Chem. Commun., 39, 1500(1974).

9. J. Farkaš and J. Škoda, Czech, Patent Appl. PV 214-73(1973). Quoted in ref. 7.

10. J. D. Warren, J. H. MacMillan and S. S. Washburne, J. Org. Chem., 40, 743(1975).

11. J. H. MacMillan and S. S. Washburne, J. Heterocyclic Chem., 12, 1215(1975).

12. H. R. Kricheldorf, Makromol. Chem., 173, 13(1973).

13. S. S. Washburne and J. H. MacMillan, unpublished results.

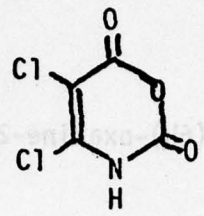
14. S. S. Washburne and H. Lee, unpublished results.

15. J. Farkaš, O. Fliegerová, and J. Škoda, Collect. Czech Chem. Commun., 41, 2059(1976).

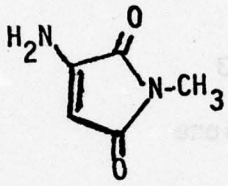
STRUCTURES OF COMPOUNDS SUBMITTED DURING REPORTING

PERIOD 74 JUNE 1 TO 76 JUNE 3D

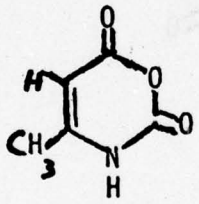
	Bottle Number	Name	Notebook Ref.
1.	BN=BE15255	4,5-dichloro-1,3-oxazine-2,6(3H)-dione	DJWI-38



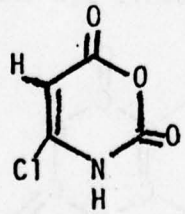
2.	BN=BE15264	2-amino-N-methylmaleimide	TFI-6



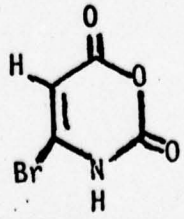
3.	BN=BE15246 BE12923	4-methyl-1,3-oxazine-2,6(3H)-dione	JDWI-31



4.	BN=BE17642 BE17651 BE17660	4-chloro-1,3(3H)-oxazine-2,6-dione	JDWI-50

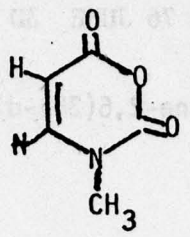


5.	BN=BE17311	4-bromo-1,3(3H)-oxazine-2,6-dione	JDWI-52

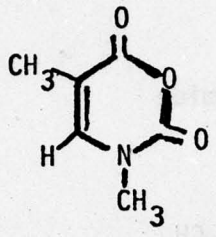


Compounds Submitted (continued...)

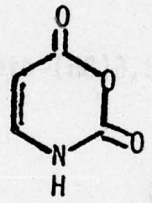
6. BN=BE17697 N-methyl-1,3(6H)-oxazine-2,6-dione 85M



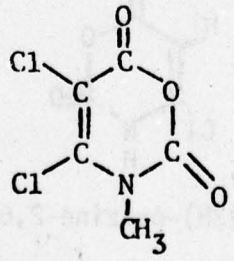
7. BN=BE17688 (4,N-) dimethyl-1,3(6H)-oxazine-2,6-dione 93M



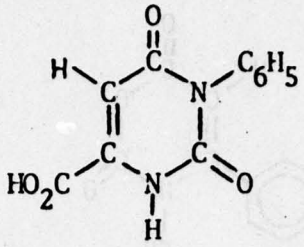
8. BN=BE10170 1,3-oxazine-2,6-dione JDWI-2



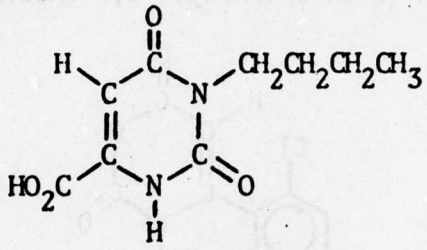
9. BN= BE43740 4,5-Dichloro-N-methyl-1,3-oxazine-2,6(3H)-dione 117M



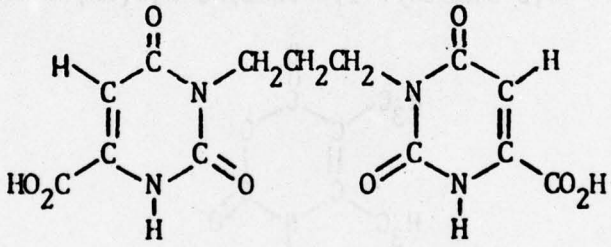
10. BN= BE50049 3-Phenylorotic acid KPII-20



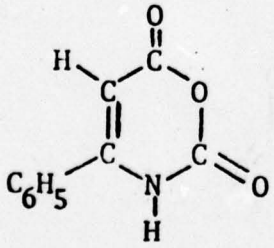
11. BN= BE50058 3-n-Butylorotic acid KPII-22



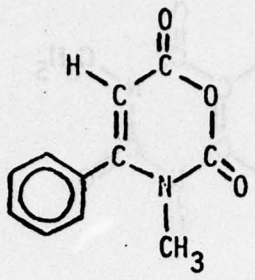
12. BN= BE50067 3,3'-trimethylenebis(orotic acid) KPII-70



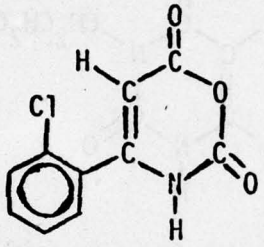
13. BN=BE66789 4-Phenyl-1,3-oxazine-2,6-dione 134M



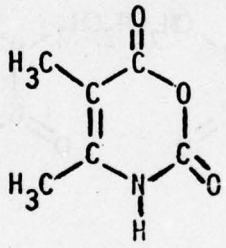
14. BN = BE66869 4-Phenyl-N-methyl-1,3-oxazine-2,6(3H)-dione (142M)



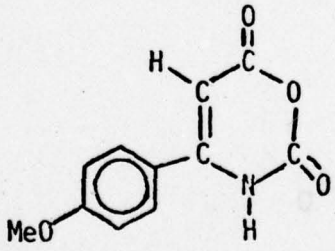
15. BN = BE57226 4-(o-Chlorophenyl)-1,3-oxazine-2,6(3H)-dione (149M)



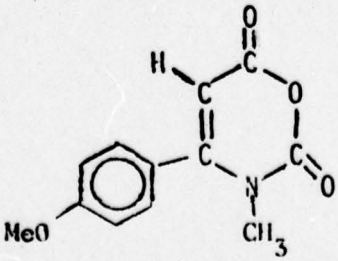
16. BN = BE58769 4,5-Dimethyl-1,3-oxazine-2,6(3H)-dione (KPIII-177)



17. BN = BE72401 4-p-anisyl-1,3-oxazine-2,6(3H)-dione (168M)

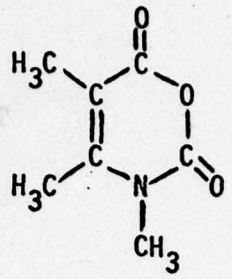


18. BN = BE72410 3-Methyl-4-p-anisyl-1,3-oxazine-2,6(3H)-dione (174M)

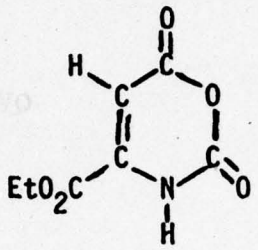


Structures (continued)

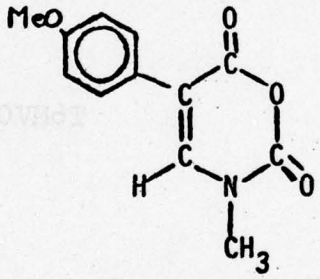
19. BN = unknown 3,4,5-Trimethyl-1,3-oxazine-2,6(3H)-dione (KPIII-178)



20. BN = BE58894 4-Carboethoxy-1,3-oxazine-2,6(3H)-dione (KPIII-179)

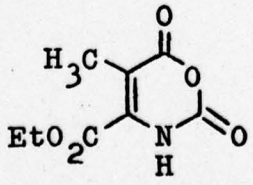


21. BN = BE76098 3-Methyl-5-p-anisyl-1,3-oxazine-2,6(3H)-dione (176M)



22. BN = BE75840 [KP-III-205]

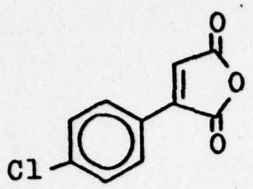
4-Carbomethoxy-5-methyl-1,3-oxazine-2,6-dione



T6MVOVJ E FV02

23. BN = BE75868 [187M]

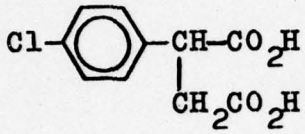
p-Chlorophenylmaleic Anhydride



T5VOVJ DR DG

24. BN = BE75877 [MLenhart]

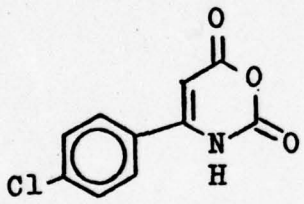
p-Chlorophenylsuccinic Acid



QV1YVQ&R DG

25. BN = BE75886 [181M]

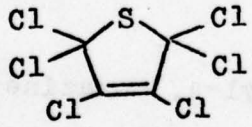
4-[p-Chlorophenyl]-1,3-oxazine-2,6-dione



T6MVOVJ FR DG

26. BN = BE75966 [CPohan]

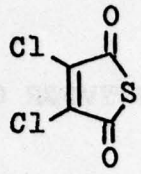
2,2,3,4,5,5-Hexachloro-3-thiolene



T5S BUJ BG BG EG EG

27. BN = BE 75975 [CPohan]

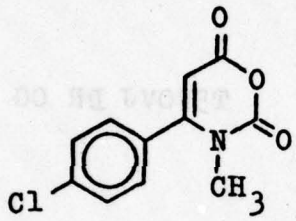
Dichlorothiomaleic Anhydride



T5VSVJ DG EG

28. BN = BE76043 [186M]

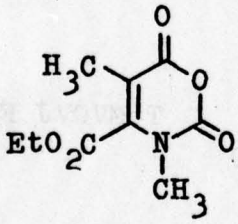
3-Methyl-4-[p-chloromethyl]-1,3-oxazine-2,6-dione



T6NVOVJ A FR DG

29. BN = BE79937 [KP-III-215]

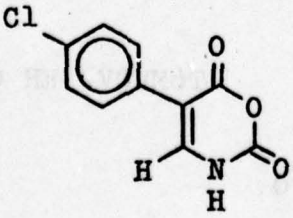
3,5-Dimethyl-4-carboethoxy-1,3-oxazine-2,6-dione



T6NVOVJ A E FV02

30. BN = BE80850 [188M]

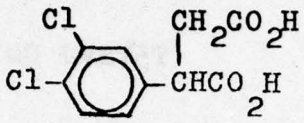
5-[p-Chlorophenyl]-1,3-oxazine-2,6-dione



T6MVOVJ ER DG

31. BN = BE85784 [MLenhart]

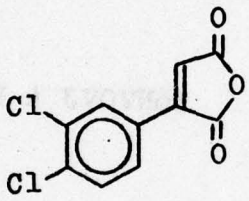
3,4-Dichlorophenyl Succinic Acid



QV1YVQ2R CG DG

32. BN = BE85793 [193M]

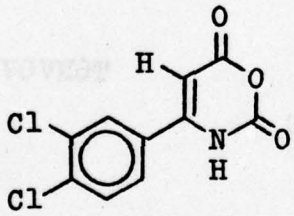
3,4-Dichlorophenylmaleic Anhydride



T5VOVJ DR CG DG

33. BN = BE85800 [194M]

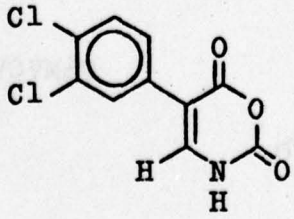
4-[3,4-Dichlorophenyl]-1,3-oxazine-2,6-dione



T6MVOVJ FR CG DG

34. BN = BE85819 [195M]

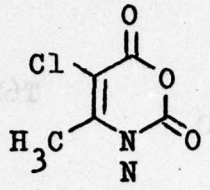
5-[3,4-Dichlorophenyl]-1,3-oxazine-2,6-dione



T6MVOVJ ER CG DG

35. BN = BE99162 [KP-III-239]

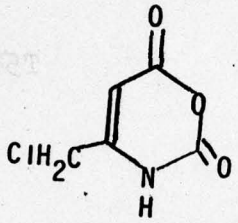
4-Methyl-5-chloro-1,3-oxazine-2,6-dione



T6MVOVJ EG F

36. BN = BE99171 [KP-III-245]

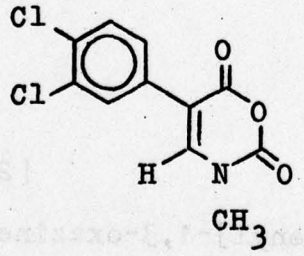
4-[Chloromethyl]-1,3-oxazine-2,6-dione



T6MVOVJ F1G

37. BN = BE99199 [200M]

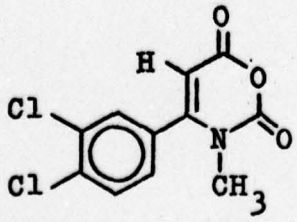
3-Methyl-5-[3,4-dichlorophenyl]-1,3-oxazine-2,6-dione



T6NVOVJ A ER CG DG

38. BN = BE99206 [198M]

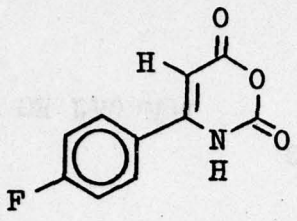
3-Methyl-4-[3,4-dichlorophenyl]-1,3-oxazine-2,6-dione



T6NVOVJ A FR CG DG

39. BN = BG00601 [2o2M]

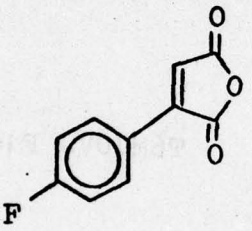
4-[p-Fluorophenyl]-1,3-oxazine-2,6-dione



T6MVOVJ FR DF

40. BN = BG03979 [203M]

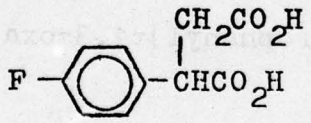
p-Fluorophenylmaleic Anhydride



T5VOVJ DR DF

41. BN = BG03997

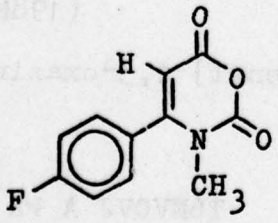
p-Fluorophenylsuccinic Acid [AM-1]



QV1YVQ&R DF

42. BN = BG04001 [207M]

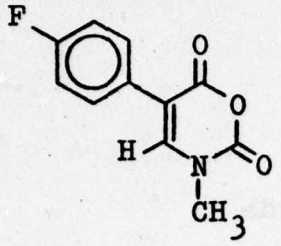
3-Methyl-4-[p-fluorophenyl]-1,3-oxazine-2,6-dione



T6NVOVJ A FR DF

43. BN = BG04010 [209M]

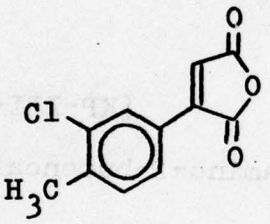
3-Methyl-5-[p-fluorophenyl]-1,3-oxazine-2,6-dione



T6NVOVJ A ER DF

44. BN = BG10670 [210M]

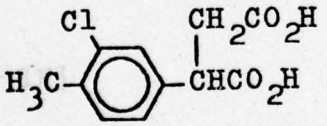
3-Chloro-4-methylphenylmaleic Anhydride



T5VOVJ DR D CG

45. BN = BG10689

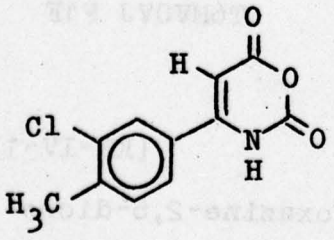
3-Chloro-4-methylphenylsuccinic Acid



QV1YVQ&R D CG

46. BN = BG11382 [211M]

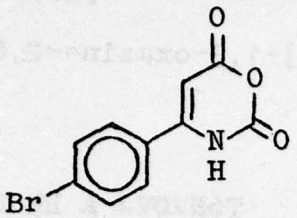
4-[3-Chloro-4-methylphenyl]-1,3-oxazine-2,6-dione



T6MVOVJ FR D CG

47. BN = BG12763

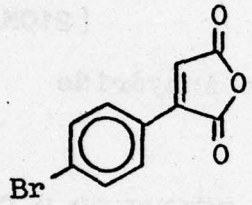
4-[p-Bromophenyl]-1,3-oxazine-2,6-dione



T6MVOVJ FR DE

48 BN = BG12722 [215M]

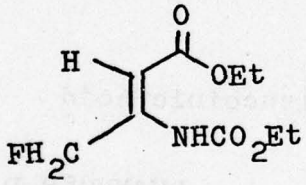
p-Bromophenylmaleic Anhydride



T5VOVJ DR DE

49. BN = BG14212 [KP-III-287, IV-3]

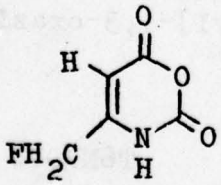
Ethyl 4-fluoro-3-carboethoxyamino-2-butenoate



20VMY1FU1V02

50. BN = BG14221 [KP-III-289]

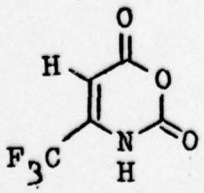
4-[fluoromethyl]-1,3-oxazine-2,6-dione



T6MVOVJ F1F

51. BN = BG37895 [KP-IV-13,15,17]

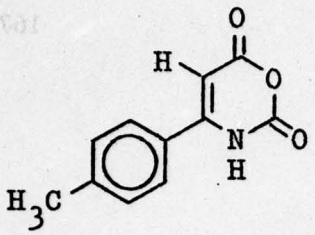
4-[trifluoromethyl]-1,3-oxazine-2,6-dione



T6MVOVJ FXFFF

52. BN = BG38043 [220M]

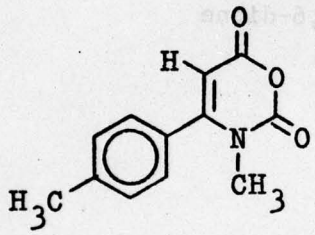
4-[p-tolyl]-1,3-oxazine-2,6-dione



T6MVOVJ FR D

53. BN = BG41559 [223M]

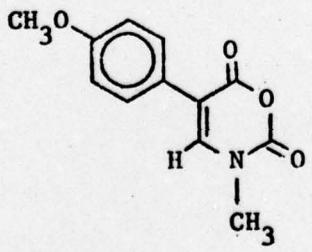
3-Methyl-4-[p-tolyl]-1,3-oxazine-2,6-dione



T6NVOVJ A FR D

54. BN = BE76098

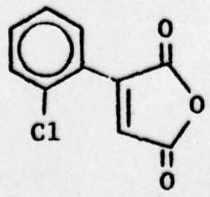
3-Methyl-5-p-anisyl-1,3-oxazine-2,6-dione



T6NVOV A ER D01

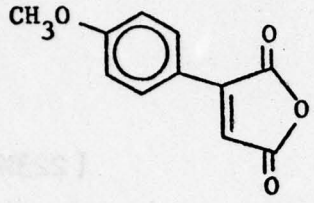
55. BN = BE79946 144M

o-Chlorophenylmaleic Anhydride



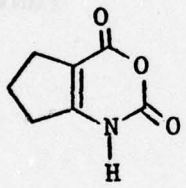
56. BN = BE79955 167M

p-Anisylmaleic Anhydride



57. BN = BE85828 KP-III-235

4,5-Trimethylene-1,3-oxazine-2,6-dione



SYNTHETIC PROCEDURES - EXPERIMENTAL DETAILS

GENERAL COMMENTS: Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 727 infrared spectrophotometer. Proton magnetic resonance spectra were obtained with Varian XL-100-15, A-60A, and Perkin-Elmer R-24B spectrometers, using tetramethylsilane as internal standard. They are reported in ppm (δ) downfield from tms. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN; and Schwartzkopf Microanalystical Laboratories, Woodside, NY. Reactions involving trimethylsilyl azide or isocyanates were carried out under dry nitrogen.

Synthetic Procedures for Submitted Compounds

1. 4,5-Dichloro-1,3-oxazine-2,6-dione—(BE15255)—Dichloromaleic anhydride (8.35 g, 50 mmol) in 20 ml of p-dioxane was refluxed with trimethylsilyl azide (5.8 g, 50 mmol) for 5 hr; 1.05 ml of gas were evolved. The solution was cooled to room temperature, filtered, treated with 3.5 ml of absolute ethanol and diluted with 50 ml of chloroform. Cooling to -20° gave 3.74 g (38%) of off-white crystals mp 209-210 (dec). Recrystallization from 1:1 ethyl acetate/hexane gave 2.2 g light yellow crystals, mp 204-206 (dec). Ir (mull) 3100(w), 1815(s), 1755(s), 1605(m), 995(m), and 895(m) cm^{-1}; nmr (DMSO-d_6) 9.9 (broad, N-H). Anal. Calcd for $C_4HCl_2NO_3$: C, 26.40; H, 0.56; Cl, 38.97; N, 7.70. Found: C, 26.48; H, 0.57; Cl, 39.07; N, 7.61.

2. 2-Amino-N-methylmaleimide—(BE15264)—To a solution of 20 mmoles of N-methylmaleimide in 40 ml of mesitylene was added 21 mmoles of trimethylsilyl

azide. The mixture, heated at reflux for 56 hr, evolved 400 ml of N_2. The reaction mixture was filtered from 0.10 g tan powder, and fractionated to afford 2.3 g (58%) of 2-trimethylsilylamino-N-methylmaleimide, yellow oil, bp 85-87°. This oil was heated a reflux 5 min in 95% ethanol. Removal of volatiles by evaporation at reduced pressure and recrystallization from benzene/heptane afforded a quantitative yeidl of 2-amino-N-methylmaleimide, yellow crystals, mp 119-121°; ir ($CHCl_3$) 3570, 3455, 1717, 1660, 1465, 1380, 1240, 1135, and 1045 cm^{-1}; nmr ($CDCl_3$) 2.75 (3H), 4.73 (1H), and 7.1 (2H, broad) ppm. Anal. Calcd for $C_5H_6N_2O_2$: C, 47.62; H, 4.80; N, 22.21. Found: C, 47.73; H, 4.89; N, 21.97.

3. 4-Methyl-1,3-oxazine-2,6-dione—(BE15246 and BE12923)—Citraconic anhydride (56.0 g, 0.5 mole) was refluxed with trimethylsilyl azide (61.0 g, 0.53 mole) in 75 ml of chloroform for 5 hr. The mixture was then cooled and treated with ca. 25 ml of 95% ethanol, which precipitated 21.0 g (33%) of microcrystalline white powder, mp 140-145° (dec). Recrystallization from ethyl acetate gave 10.6 g of 4-methyloxazinedione, mp 176-177°; ir (mull) 3300, 3125, 1790, 1710, 1640, 1040, and 970 cm^{-1}; nmr (DMSO-d_6) 2.08 (s,3), 5.38 (s,1), and 11-12 (broad, 1) ppm; Mass spectrum (70 v) m/e (rel. intensity) 127 (57.2)M^+; 83 (46); 68 (46); 44 (41.3); and 42 (100); Anal. Calcd for $C_5H_5NO_3$: C, 47.25; H, 3.96; N, 11.02. Found: C, 47.37; H, 3.98; N, 11.16.

4. 4-Chloro-1,3-oxazine-2,6-dione—(BE17642, BE17651, and BE17660)—Chloromaleic anhydride (6.0 g, 70% by weight commercial material, 32 mmol) and trimethylsilyl azide (11.0 g, 95 mmol) were heated cautiously to 70-90° for one hr. Dilution with 40 ml of benzene, cooling, and treatment with a

stoichiometric amount of absolute ethanol precipitated 2.7 g of tan powder mp 133.5-134.5° (dec). Sublimation at 100° (0.2 mm) afforded 1.43 g (29%) of white powder mp 135-137° (dec). Recrystallization of this powder from ethyl acetate gave 0.40 g of white needles, mp 139-140° (dec); ir (mull) 3120, 1790, 1725, 1615, 1120, and 980 cm^{-1}; nmr (acetone-d_6) 5.84 (s,1) and 11.0 (broad s,1) ppm. Anal. Calcd for $C_4H_2ClNO_3$: C, 32.57; H, 1.37; N, 9.49; Cl, 24.03. Found: C, 32.66; H, 1.42; N, 9.56; Cl, 24.11.

5. 4-Bromo-1,3-oxazine-2,6-dione—(BE17311)—Bromomaleic anhydride (8.85 g, 50 mmol) and trimethylsilyl azide (8.9 g, 77 mmol) were reacted at 70-90° for one hour as described for chloromaleic anhydride above. Workup in similar fashion and repeated recrystallization from hot ethyl acetate gave an analytical sample, mp 149-151° (dec); ir (mull) 3175, 1780, and 1602 cm^{-1}; nmr (DMSO-d_6) 5.87 (s,1) and 11.0 (broad, 1) ppm. Anal. Calcd for $C_4H_2BrNO_3$: C, 25.03; H, 1.05; Br, 41.63; N, 7.30. Found: C, 25.02; H, 1.00; Br, 41.48; N, 7.37. The crude reaction mixture contains approximately 25% of the 5-bromo isomer, which must be removed by repeated recrystallization.

6. N-Methyl-1,3-oxazine-2,6-dione—(BE17697)—A solution of 2.5 g (22 mmol) of 1,3-oxazine-2,6-dione in 65 ml of acetone was refluxed with 3.2 g (25 mmol) of dimethyl sulfate and 2.5 g (30 mmol) of sodium bicarbonate for 20 hr. The mixture was filtered hot, and acetone removed from the filtrate under reduced pressure. The remaining semi-solid residue was triturated with hot ethyl acetate. Cooling gave 2.0 g (71%) of N-methyloxazinedione as colorless crystals, mp 110-111° (dec); ir ($CHCl_3$) 3130, 1790, 1745, 1715, 1640, and 1360 cm^{-1}; nmr (DMSO-d_6) 3.2 (s,3), 5.65 (d,1); 7.75 (d,1); mass spectrum (70 v) m/e (rel. intensity) 127 (100) M^+, 83 (132), 55 (109),

44 (70), 42 (190). Anal. Calcd for $C_5H_5NO_3$: C, 47.25; H, 3.96; N, 11.02. Found: C, 47.01; H, 3.90; N, 10.91.

7. 4,N-Dimethyl-1,3-oxazine-2,6-dione—(BE17688)—A mixture of 1.7 g (13.3 mmol) of 4-methyl-1,3-oxazine-2,6-dione in 40 ml of acetone was refluxed with 1.9 g (15 mmol) of dimethyl sulfate and 1.5 g (18 mmol) of sodium bicarbonate for 22 hr. Workup as described for compound 6. afforded 1.2 g (64%) of 4,N-diemthyloxazinedione, colorless crystals, mp 83-85° (dec); ir ($CHCl_3$) 3120, 2960, 1780, 1720, 1630, and 1370 cm^{-1}; nmr (DMSO-d_6) 2.4 (d,3); 3.4 (s,3); and 5.6 (q,1) ppm. Anal. Calcd for $C_6H_7NO_3$: C, 51.07; H, 4.99; N, 9.92. Found: C, 50.90; H, 5.23; N, 9.78.

8. 1,3-Oxazine-2,6-dione—(BE10170)—Maleic anhydride (4.8 g, 49 mmol) in 15 ml of deuteriochloroform was refluxed with trimethylsilyl azide for one hour. Dilution with 20 ml of benzene and hydrolysis with ethanol gave 3.81 g (69%) of off-white powder, mp 158-159° (dec); ir (mull) 3300, 3150, 3120, 1790, 1710, 1635, 1200, 1105, 1055, and 980 cm^{-1}; nmr (DMSO-d_6) 5.56 (d,1, J=7.5 Hz), 7.52 (d,1,J=7.5 Hz), and 10.75 (broad, 1) ppm; mass spectrum (70 v) m/e (rel. intensity) 113 (58)M^+, 69 (100), 43 (52), and 44 (52). This reaction is best run on a small scale as described. Scale-up led to lower yield, probably because of inefficient temperature control. An illustrative procedure, optimized after the termination of the contract period is as follows—

A flame-dried 50 ml three-neck flask is charged under nitrogen with 4.9 g (50 mmol) of maleic anhydride and 14 ml (0.1 mol) of trimethylsilyl azide.

A thermometer is inserted and the slurry gently heated with stirring. At about 40° the anhydride dissolves and gas evolution commences. The reaction gently exotherms at this point and external heating is discontinued. Gas evolution becomes moderate at 55-60°. With the aid of a water bath the reaction temperature is maintained at 55-60°. The temperature must not be allowed to exceed 60°. After three hours heating, gas evolution ceases and the reaction is cooled to room temperature, diluted with 30 ml of chloroform, then treated with 2.5 g (54 mmol) of absolute ethanol. Cooling to 0° gives white micorcrystals of oxazinedione which are collected and washed with ether. The yield 3.2 g (57%), mp 158-162°. Partial concentration and cooling of the filtrate gives a second crop, 1.2 g, mp 158-160° of tan micro crystals; total yield 4.4 g (78%).

This modified procedure has not been attempted for other oxazinediones, but probably will increase the yield measurably.

Synthetic Routes to Compounds Submitted

9. 4,5-Dichloro-N-methyl-1,3-oxazine-2,6-dione—(BE43740)—A solution of 2.6 g (14.3 mmol) of 4,5-dichloro-1,3-oxazine-2,6-dione-(BE15255)-in 25 ml of acetone was refluxed under nitrogen with 2.9 g (23 mmol) of dimethyl sulfate and 1.5 g (18 mmol) of sodium bicarbonate for three hours. The hot solution was filtered, and the filtrate cooled to 0° giving 2.0 g (71%) of BE43740, as white crystals, mp 171-173° (dec). Ir ($CDCl_3$) 1810, 1800, 1750, 1595, 1430, 1375, 1300, 1055, and 1005 cm^{-1}; pmr (DMSO-d_6) δ3.68 (s,CH_3-N) ppm. Anal Calcd for $C_5H_3NO_3Cl$: C, 30.64; H, 1.54; N, 7.15; Cl, 36.18. Found: C, 30.49; H, 1.49; N, 7.13; Cl, 36.05.

10. 3-Phenylorotic Acid—(BE50049)—A mixture of 30 mmol of phenyl isocyanate and 15 mmol of diethylaminofumarate were heated with 0.1 g of anhydrous aluminum chloride for 1.5 hr at 120°. The reaction mixture solidified on cooling, and was recrystallized from ethanol/water to afford 49% of 3-phenyl-5-carboethoxymethylidenehydantoin, mp 191°. The hydantion was heated on a steam bath with 7.5 ml of 2 N potassium hydroxide solution and 4 ml of ethanol for 16 hr, then acidified with 2N hydrochloric acid. The precipitate was collected by filtration and recrystallized from ethanol/water to afford 84% of BE50049, mp 280° (dec). Ir 1710, 1660, and 1640 cm^{-1}; pmr δ7.2 (5,m), 6.26 (1,s) ppm.

11. 3-n-Butylorotic Acid—(BE50058)—A reaction sequence as in II. starting from n-butylisocyanate gave a 58% overall yield of BE50058, mp 197-198°. Ir 1730, 1710, 1660, 1640 cm^{-1}; pmr δ6.14 (1,s,C=C-H) plus peaks due CO_2H and an n-butyl group. Anal. calcd for $C_9H_{12}O_4N_2 \cdot H_2O$: C, 46.96; H, 6.13; N, 12.17. Found: C, 47.09; H, 6.16; N, 12.18.

12. 3,3'-Trimethylenebis(orotic acid)—(BE50067)—A reaction sequence as in II. starting from 50 mmol of trimethylenebis(isocyanate) and 100 mmol of diethyl aminofumarate gave 16% of the hydantoin, which was converted in 91% yield to BE50067, mp 310° (dec). Ir (mull) 3400-2200 (broad), 1735, 1645, 1375, 1255, 1190, 745 cm^{-1}; pmr δ6.13 (2,s,C=C-H) plus peaks due to CO_2H and a trimethylene moiety. Anal. calcd for $C_{13}H_{12}N_4O_8 \cdot H_2O$: C, 42.17; H, 3.81; N, 15.13. Found: C, 42.41; H, 3.62; N, 15.23.

13. 4-Phenyl-1,3-oxazine-2,6-dione—(BN unknown)—A mixture of 4.4 g (25 mmol) of phenylmaleic anhydride (prepared by the method of R. K. Hill) was heated at reflux with 3.5 g (30 mmol) of trimethylsilyl azide in 5 ml of p-dioxane. After 4 hr heating, c. 500 ml of gas had been evolved. The mixture was cooled to -5° and sequentially treated with 25 ml of chloroform and 1.4 g absolute ethanol, precipitating 1.7 g (36%) of 4-phenyl-1,3-oxazine-2,6-dione, mp 198-199° (dec). Ir (mull) 3210, 3170, 3100, 1810, 1710, 1630, 1500, 1280, 1260, 1120, 1080, 980, 840, 740, and 680 cm^{-1}; pmr (DMSO-d_6) δ6.1 (s,1,C=C-H), 7.7 (m,5,phenyl), and 11.0 (broad, 1,N-H) ppm. Anal. calcd for $C_{10}H_7NO_3$: C, 63.49; H, 3.73; N, 7.40. Found: C, 63.47; H, 3.65; N, 7.40.

Synthetic Routes to Compounds Submitted

14. 4-Phenyl-N-methyl-oxazinedione—(BE66869)—A mixture of 0.8 g (4 mmol) of 4-phenyl-1,3(3H)-oxazine-2,6-dione, 15 ml of acetone and 1.0 g (8 mmol) of dimethyl sulfate were heated at reflux under a nitrogen atmosphere for 23 hr, at which time tlc analysis (silica gel-EtOAc eluent) indicated completeness of the reaction. The solution was filtered hot and removal of acetone gave a yellow semi-solid, which was tritutated with 5 ml of hot acetate. Cooling afforded 0.4 g (46.5%) of the desired N-methyl derivative (BE66869), mp 118120° (dec). Recrystallization from EtOAc gave an analytical sample, mp 120-122° (dec). Ir spectrum ($CDCl_3$) 3120, 3070, 2970, 1790(s), 1720(s), 1630(s), 1600, 1440, 1410(s), 1320(s), 1210, 1180, 1090, 1010, 960, and 805 cm^{-1}; nmr (DMSO-d_6) δ7.3 (s,5,C_6H_5), 5.28 (s,1,H-C=C),

and 3.10 (s,3,N-CH_3) ppm. Anal. calcd for $C_{11}H_9NO_3$: C, 65.02; H, 4.46; N, 6.89. Found: C, 65.15: H, 4.40; N, 6.85.

15. 4-(o-Chlorophenyl)-oxazinedione (BE57226)--o-Chlorophenylmaleic anhydride (1.7 g, 8 mmol) was heated at reflux with 5 ml (ca. 36 mmol) of trimethylsilyl azide for 2.5 hr, at which time the theoretical amount of nitrogen (200 ml) had been evolved. The solution was cooled to 0° and 0.4 ml of absolute ethyl alcohol added, causing separation of some solid. Tlc analysis (silica gel-EtOAc eluent) showed two spots of nearly equal intensity. Removal of volatile material from the solution left 1.8 g of brown semi-solid, which was dissolved in 30 ml of chloroform and extracted with 4 x 10 ml of saturated aqueous sodium bicarbonate, then 4 x 10 ml of water. After drying ($MgSO_4$) and evaporation of the chloroform, 1.0 g of a pasty semi-solid was obtained whose pmr spectrum indicated a 55%/45% mixture of 4- and 5-o-chlorophenyl oxazinediones. The semi-solid was dissolved in 10 ml of chloroform, cooled to 0°, and treated dropwise with n-hexane to a point of permanent turbidity. Cooling afforded 0.20 g (11%) of pure 4-o-chlorophenyl-oxazinedione (BE57226). Ir spectrum ($CDCl_3$) 3240, 1800(s), 1730(vs), 1640(s), 1590, 1350, 1310, 1080, 1050, 1020, 970(s), and 805 cm^{-1}; nmr (DMSO-d_6) δ5.80 (s,1,C_5-H), 7.50 (m,4,atomatic), and 9.2 (br,1,N-H, seen only in integration) ppm. The 5-ochlorophenyl isomer could be obtained contaminated with ca 10% 4-isomer by further workup of the mother liquors. Anal. (obtained on a mixture of 67% 4-isomer and 33% 5-isomer) calcd for $C_{10}H_6ClNO_3$: C, 53.71; H, 2.70; N, 6.26; Cl, 15.85. Found: C, 53.51; H, 2.59; N, 6.52; Cl, 16.03.

16. 4,5-Dimethyl-oxazinedione—(BE58769)—A mixture of 3.0 g (20 mmol) of ethylmethylacetoacetate (Aldrich) and 1.8 g (20 mmol) of ethyl carbamate in 10 ml of phosphoryl chloride was heated at 70-75° for 2.5 hr. Hydrogen chloride was evolved. Volatiles were removed by water-pump evaporation and finally Kugel-rohr distillation (0.05 mm). The black residue was treated with 40 ml of chloroform, then extracted with 3 x 40 ml of cold saturated aqueous sodium bicarbonate and 1 x 40 ml of water. The combined layers were extracted with 4 x 100 ml of ethyl acetate, then the combined ethyl acetate layers were dried (Na_2SO_4) and evaporated to give crude crystalline material, which was recrystallized from chloroform to afford 1.2 g (43%) of dimethyl-oxazinedione (BE58769), mp 130-135°, which, by estimation from the pmr spectrum, was contaminated with 5% of ethyl allophanate. Sublimation (50-55°, 0.05 mm) caused the allophanate to collect on the cold finger. The oxazinedione remaining has mp 143-145°. Ir spectrum (mull) 3240, 3190, 1780(sh), 1760(s), 1730(s), 1715(s), and 1650(s) cm^{-1}; nmr (DMSO-d_6) δ11.14 (br,s,1,N-H), 2.08 (s,3,4-Me), and 1.83 (s,3,5-Me) ppm. Anal. calcd for $C_6H_7NO_3$: C, 51.07; H, 5.00; N, 9.93. Found: C, 50.98; H, 4.87; N, 9.85.

17. 4-(p-Anisyl)-oxazinedione—(BE72401)—Under a nitrogen atmosphere, 7 g (34 mmol) of p-anisylmaleic anhydride was added with stirring to 12 ml of p-dioxane. Trimethylsilyl azide (10 ml, 75 mmol) was added and the resultant solution heated at reflux for 3 hr, after which time ca. 900 ml of nitogen had been evolved. The solution was cooled to 0° and 80 ml of benzene added, then stirred 5 min. Absolute ethanol (2 g, 43 mmol) was added, causing precipitation of a yellowish solid, which was collected by suction

filtration, washed with benzene, and dried, affording 4.3 g (58%) of crude oxazinediones, estimated by nmr to be 70% 4- and 30% 5-p-anisyl-oxazinedione (20% yield, mp 199-201° (dec), off-white microcrystals. Ir spectrum ($CDCl_3$) 3350, 3120, 2910, 1790(vs), 1720(d,s), 1630, 1605, 1560, 1520, 1280, 1250, 1210, 1170, 1080(s), 1030, and 980(s) cm^{-1}; nmr (DMSO-d_6) δ11.6 (br,s,1,N-H), 7.85 (d,2,J=9), 7.10 (d,2,J=9), 5.95 (s,1,C_5-H), and 3.90 (s,3,OCH_3) ppm. Anal. calcd for $C_{11}H_9NO_4$: C, 60.27; H, 4.14; N, 6.39. Found: C, 60.0; H, 4.08; N, 6.32.

18. 3-Methyl-4-(p-anisyl)-oxazinedione—(BE72410)—A solution of 1.0 g (4.6 mmol) of 4-(p-anisyl)-oxazinedione in 20 ml of acetone was heated at reflux with 1.8 g (14 mmol) of dimethyl sulfate and 1.0 g (12 mmol) of sodium bicarbonate until tlc analysis (silica gel-EtOAc) indicated complete reaction (24 hr). The hot solution was filtered. Removal of acetone from the filtrate left a semi-solid residue which was triturated with 15 ml of hot ethyl acetate. Cooling afforded 0.67 g (63%) of white crystals in five crops: 3-methyl-4-anisyl oxazinedione (BE72410) mp 158-159° (dec). Ir spectrum ($CDCl_3$) 3030, 2990, 2950, 2860, 1780(vs), 1720(vs), 1620(s), 1610(s), 1570, 1520(s), 1435(s), 1320(s), 1300(s), 1250(s), 1210, 1170(s), 1090, 1070, 1020, 1005, 970, 830(s), and 810(s) cm^{-1}; nmr (DMSO-d_6) δ7.45 (d,2,J=9), 7.05 (d,2,J=9), 5.55 (s,1,C_5-H), 8.80 (s,3,OCH_3), and 3.10 (s,3,N-CH_3) ppm. Anal. calcd for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.70; H, 4.73; N, 5.91.

19. 3,4,5-Trimethyl-oxazinedione—(BE unkown, KP-III-178)—A mixture of 0.99 g (7 mmol) of 4,5-dimethyloxazinedione (BE58769), 1.26 g (10 mmol) of dimethyl sulfate, and 0.67 g (8 mmol) of sodium bicarbonate in 15 ml of

acetone was heated at reflux until tlc analysis indicated complete reaction (72 hr). The hot solution was filtered, the filtrate concentrated to a yellow semi-solid, and the residue recrystallized from ethyl acetate to give 0.52 g (40%) of trimethyl-oxazinedione, mp 119-121°. Ir spectrum (mull) 1770(vs), 1720(s), 1630, 1420, 1320, and 735(s) cm^{-1}; nmr ($CDCl_3$) δ3.39 (s,3,N-CH_3), 2.23 (s,3,4-CH_3), and 1.95 (s,3,5-CH_3) ppm. Anal. calcd for $C_7H_9NO_3$: C, 54.19; H, 5.85; N, 9.03. Found: C, 53.98; H, 6.00; N, 8.97.

20. 4-Carboethoxyl-oxazinedione—(BE58894)—A mixture of 3.76 g (20 mmol) of diethyl oxaloacetate (Aldrich) and 1.80 g (20 mmol) of ethyl carbamate (Aldrich) in 10 ml of phosphoryl chloride was heated at 90-100° for 2.5 hr. Phosphoryl chloride was removed by evaporation at reduced pressure and finally by distillation (Kugel-rohr, 0.05 mm). The red residue was treated with 50 ml of benzene, and extracted with 4 x 50 ml of water. The combined aqueous layers were extracted with 3 x 50 ml of ethyl acetate. The combined ethyl acetate extracts were dried (Na_2SO_4), evaporated, and recrystallized from ethyl acetate to afford 0.8 g (22%) of ethyl oxaorotate (BE58894), mp 138-140° (dec). Ir Spectrum (mull) 3240, 3190, 3130, 1805(s), 1745(vs), 1730(vs), 1710(vs), 1650, and 1495 cm^{-1}; nmr ($CDCl_3$) δ8.5 (br,s,1,N-H), 6.31 (s,1,C_5-H), 4.45 (q,2,O-CH_2-CH_3), and 1.40 (t,3,O-CH_2-CH_3) ppm. Anal. calcd fro $C_7H_7NO_5$: C, 45.41; H, 3.81; N, 7.57. Found: C, 45.63; H, 3.90; N, 7.71.

Further workup of the benzene layer above afforded 0.45 g (9%) of ethyl 3-carboethoxy-orotate. This material was procuded in greater yield when the reaction was carried out at temperatures above 100°, while a temperature of 60-65° produced principally N-ethoxycarbonylaminomaleic anhydride.

21. 3-Methyl-5-(p-anisyl)-oxazinedione—(BE76098)—A mixture of isomeric oxazinediones (4- and 5-p-anisyl ca. 1:1, see cpd IV, BE72401 above) (1.83 g, 8.4 mmol) was heated at reflux with 4.0 g (32 mmol) of dimethyl sulfate, 2.0 g (24 mmol) of sodium bicarbonate, and 35 ml of acetone until tlc analysis (silica gel-EtOAc eluent) indicated complete reaction (24 hr) to two isomeric N-methyl compounds. The hot solution was filtered and then concentrated to a semi-solid residue, which was triturated with hot ethyl acetate. On cooling, 0.47 g of pure 3-methyl-5-(p-anisyl)oxazinedione separated, mp 159-161 dec. Further amounts of BE76098 could be obtained by column chromatography (silica gel, 1:1 chloroform/ethyl acetate). Total yield 0.54 g (55%). Ir spectrum ($CDCl_3$) 3020, 2990, 2910, 2850, 1790(s), 1730(s), 1640(s), 1610, 1515, 1330(s), 1290, 1250(s), 1230, 1180, 1150, 1080(s), 1030, 1010, 975, and 820(s) cm^{-1}; nmr (DMSO-d_6) δ8.0 (s,1, C_4-H), 7.50 (d,2,j=9), 6.95 (d,2,J=9), 3.80 (s,3,O-CH_3), and 3.40 (s,3, N-CH_3) ppm. Anal. calcd for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.74; H, 4.68; N, 5.91.

22. 4-Carboethoxy-5-methyl-2H-1,3-oxazine-3H-2,6-dione—(BE75840, KP-III-205) A mixture of 8.0 g (40 mmol) of diethyl oxalpropionate (Aldrich) and 3.6 g (40 mmol) of ethyl carbamate in 20 ml of phosphoryl chloride was heated at 100-100° for 2.5 hours. Phosphoryl chloride was removed by rotary evaporation at water-pump pressure and then by Kugel-rohr under high vacuum. The residue was triturated with anhydrous ether. The precipitated solid was collected and recrystallized from ethyl acetate. This gave 2.1 g (26%) of the tital compound, mp 94-95.5°: Ir (mull) 3230 (w), 3180(m), 1800(s), 1740(vs), 1720(vs), 1640(m), 1280(s) and 1000(s) cm^{-1}. Nmr ($CDCl_3$) δ8.63

(broad s,1), 4.47 (q,2,J=7 Hz). Anal. calcd for $C_8H_9NO_5$: C, 48.25; H, 4.56; N, 7.03. Found: C, 48.18; H, 4.66; N, 7.16.

23. p-Chlorophenyl maleic anhydride—(BE75868, Ref. 187 M)—This compound, previously reported[1], was prepared by the literature[2] procedure.

24. p-Chlorophenyl succinic acid—(BE75877, Ref. M. Lenhart)—This compound was prepared by the literature[3] procedure.

25. 4-(p-Chlorophenyl)-2H-1,3(3H)-oxazine-2,6-dione—(BE75886, Ref. 181M)—p-Chlorophenylmaleic anhydride[1] (3.5 g 16.8 mmol) was refluxed with 10 ml of trimethylsilyl azide (∿75 mmol) and 5 ml p-dioxane for four hours. Standard workup gave 1.5 g (37%) of isomeric oxazinedione mp 185-90° (dec). Crystallization from ethyl acetate gave 810 mg of pure 4-isomer mp 207-9° (dec). Ir (mull) 3230(w), 3160(w), 3120(w), 1790(s), 1710(s), 1630(s), 1595(m), 1560(m), 1505(m), 1370(s), 1270(m), 1250(m), 1110(m), 1090(m), 1010(m), 970(m), 840(m), 820(m), 810(m), 750(m), 710(m) cm^{-1}. Pmr (DMSO-d_6) δ11.0 (s,broad,N-H), 7.2 (AB pattern,4,aromatics), 5.6 (s,1,C_5-H) PPM. Anal. calcd for $C_{10}H_6ClNO_3$: C, 53.71; H, 2.70; N, 6.26; Cl, 15.85. Found: C, 53.89; H, 2.78; N, 6.20; Cl, 15.74.

26. 2,2,3,4,5,5-Hexachloro-3-thiolene—(BE75966, Ref. C. Pohan)—This compound was prepared by the literature[4] procedure.

27. Dichlorothiomaleic anhydrice—(BE75975, Ref. C. Pohan)—This compound was prepared by the literature[5] procedure.

28. 3-Methyl-4-(p-chlorophenyl-2H-1,3(3H)-oxazine-2,6-dione—(BE76043, Ref. 186 M)—4-(p-Chlorophenyl) oxazinedione (760 mg, 3.4 mmol) was refluxed with

dimethylsulfate (700 mg, 5.6 mmol) and sodium bicarbonate (340 mg, 4 mmol) in acetone (20 ml) for 42 hours. Standard workup gave 565 mg of 3-methlated product (70%), mp 163-5° (dec). Ir ($CDCl_3$) 3120(w), 1790(vs), 1730(vs), 1625(s), 1595(m), 1495(s), 1430(s), 1405(s), 1320(s), 1210(m), 1180(m), 1080(s), 1060(m), 1020(m), 1005(s), 960(m), 820(s), 805(s) cm^{-1}. Pmr ($CDCl_3$) δ7.40 (AB pattern, 4, aromatics), 5.56 (s,1,C_5-H), 3.33 (s,3,N-CH_3) PPM. Anal. calcd for $C_{11}H_8ClNO_3$: C, 55.60; H, 3.39; N, 5.89; Cl, 14.92. Found: C, 55.52; H, 3.34; N, 5.83; Cl, 14.85.

29. Preparation of 3,5-dimethyl-4-carboethoxy-2H-1,3-oxazine-3H-2,6-dione—(BE79937, KP-III-215)—A mixture of 1.60 g (8mmole) of 5-methyl-4-carboethoxyoxazinedione, 1.51 g (12 mmole) of dimethyl sulfate and 0.84 g (10 mmole) of sodium bicarbonate in 25 ml of anhydrous acetone was heated at reflux 18 hours, at which time tlc (hexane 2: ethyl acetate 1 eluent) showed that the reaction was complete. Sodium bicarbonate was removed by filtration and the filtrate was concentrated to give a solid. This solid was recrystallized from ether-ethyl acetate to yield 1.3 g (76%) of the title compound, mp 79.5-81°. Ir (mull) 1785(s), 1740(s), 1725(vs), 1650(s), 1325(s), 1270(s), 1230(m), 770(m), and 735(s) cm^{-1}. Nmr ($CDCl_3$) δ4.46 (q,2, J=7 Hz), 3.29 (s,3,-NCH_3), 1.90 (s,3,5-Me) and 1.41 (t,3,J=7 Hz). Anal. calcd for $C_9H_{11}NO_5$: C, 50.71; H, 5.20; N, 6.57. Found: C, 50.67; H, 5.25; N, 6.50.

30. 5-(p-Chlorophenyl)-2H-1,3(3H)-oxazine-2,6-dione—(BE80850, Ref. 188 M)—The pure 5-isomer could be isolated careful fractional crystallization of the mother liquors from Ref. 186 M above. The yellow-white crystalline solid had mp 195-7° (dec). Ir (mull) 3280(m), 3160(m), 1780(s), 1710(s), 1630(s), 1260(m), 1160(m), 1080(m), 990(m), 970(m), 930(m), 810(m), 740(m) cm^{-1}.

Pmr (DMSO-d_6) δ10.8 (broad,1, seen only in integration, N-H), 7.65 (s,1, C_4-H), 7.27 (AB pattern, 4, aromatics) PPM. Anal. calcd for $C_{10}H_6ClNO_3$: C, 53.71; H, 2.70; N, 6.26; Cl, 15.85. Found: C, 53.79; H, 2.71; N, 6.26; Cl, 15.81.

31. 3,4-Dichlorophenyl succinic acid—(BE85784, Ref. M. Lenhart)—This compound, previously reported[6], was synthesized by the literature[3] procedure.

32. 3,4-Dichlorophenyl maleic anhydride—(BE85793, Ref. 193 M)—This compound, previously reported[7], was synthesized by the literature[2] procedure.

33. 4-(3,4-Dichlorophenyl)2H-1,3(3H)-oxazine-2,6-dione—(BE85800, Ref. 194 M)—3,4-Dichlorophenylmaleic anhydride[7] (7.7 g, 31.7 mmol) was heated at reflux with trimethylsilyl azide (14 ml, 0.1 mol) and p-dioxane (3.5 ml) for four hours. Standard workup gave 4.1 g (50%) of isomeric mixture of oxazinediones mp 155-65° (dec). Crystallization from ethyl acetate gave 1.01 g of pure 4-isomer, mp 199-201° (dec). Ir (mull) 3200(m), 1790(s), 1710(s), 1630(s), 1120(m), 1100(m), 1070(m), 1010(m), 960(s), 870(m), 840(m), 740(m) cm^{-1}. Pmr (DMSO-d_6) δ11.0 (broad, 1, N-H), 7.60 (s,1, aromatic), 7.33 (s,2, aromatic), 5.75 (s,1,C_5-H) PPM. Anal. calcd for $C_{10}H_5Cl_2NO_3$: C, 46.54; H, 1.95; N, 5.42; Cl, 27.48. Found: C, 46.40; H, 2.05; N, 5.39; Cl, 27.42.

34. 5-(3,4-Dichlorophenyl)-2H-1,3(3H)-oxazine-2,6-dione—(BE85819, Ref. 195 M Careful fractional crystallization of the mother liquor from Ref. 194 M above gave 640 mg of pure 5-isomer, mp 203-5° (dec). Ir (mull) 3260(w), 3180(w), 1780(s), 1720(s), 1630(s), 1340(s), 1280(m), 1240(m), 1150(m), 1120(w), 1040(w), 1010(m), 990(m), 980(m), 950(m), 920(w), 870(m), 820(m), 740(s), 660(s) cm^{-1}. Pmr (DMSO-d_6) δ10.0 (broad, 1, N-H), 7.56 (s,1,C_4-H), 7.40

(t,1,J=1Hz, aromatic), 7.15 (d,2,J=1Hz, aromatic) PPM. Anal. calcd for $C_{10}H_5Cl_2NO_3$: C, 46.54; H, 1.95; N, 5.42; Cl, 27.48. Found: C, 46.48; H, 1.96; N, 5.38; Cl, 27.43.

35. Preparation of 4-methyl-5-chloro-2H-1,3-oxazine-3H-2,6-dione--(BE99162, KP-III-239)--A mixture of 6.0 g (40 mmole) of ethyl 2-chloroacetoacetate (Aldrich) and 3.6 g (40 mmole) of ethyl carbamate in 20 ml of phosphoryl chloride was heated at 80-85° for 2.5 hours. After removing phosphoryl chloride under reduced pressure, 60 ml of chloroform was added to the yellowish residue. This resuidue was extracted with disstilled water (1x100 ml, 4x50 ml). Combined aqueous layers were extracted with ethyl acetate (2x100 ml, 3x50 ml). The ethyl acetate layers were combined, dried over sodium sulfate and concentrated to yield 1.4 g (22%) of the title compound as a white solid after trituration of the residue with ether, mp dec. >140°: Ir (solution in $CDCl_3$) 3160(m), 1790(s), 1725(vs), and 1635(m) cm^{-1}: Ir (mull) 3200(m), 1815(m), 1785(s), 1725(vs), 1705(s), 1635(m), and 1620(m) cm^{-1}. Nmr (DMSO-d_6-$CDCl_3$) δ11.92 (broad s,1), 2.23 (s,3). Anal. calcd for $C_5H_4ClNO_4$: C, 37.18; H, 2.50; N, 8.67; Cl, 21.95. Found: C, 37.30; H, 2.53; N, 8.76; Cl, 21.82.

36. Preparation of 4-chloromethyl-2H-1,3-oxazine-3H-2,6-dione--(BE99171, KP-III-245)--A mixture of 6.6 g (40 mmole) of ethyl 4-chloroacetoacetate (Aldrich) and 3.6 g (40 mmole) of ethyl carbamate in 20 ml of phosphoryl chloride was heated at 60-65° for 1.5 hours and then 85-90° for 1.5 hours. After removing phosphoryl chloride under reduced pressure, 60 ml of chloroform was added to the red-black residue. This was extracted with distilled

water (1x100 ml, 5x50 ml). The combined aqueous solutions were extracted with ethyl acetate (2x100 ml, 6x50 ml). The ethyl acetate layers were combined, dried over sodium sulfate and concentrated to afford yellow crystalline solid. After trituration of the solid with ether, 1.9 g (30%) of the title compound was obtained, mp 135-138° (dec). Ir (mull) 3250(m), 3120(w), 1785(s), 1725(vs), and 1640(s) cm^{-1}. Nmr (DMSO-d_6-$CDCl_3$) δ11.78 (broad s,1), 5.74 (s,1) and 4.34 (s,2). The analytical sample was prepared by recrystallization from ether-ethyl acetate, mp 140-142° (dec). Anal. calcd for $C_5H_4ClNO_3$: C, 37.18; H, 2.50; N, 8.67; Cl, 21.95. Found: C, 37.25; H, 2.58; N, 8.59; Cl, 21.79.

37. 3-Methyl-5-(3,4-dichlorophenyl)-2H-1,3(3H)-oxazine-2,6-dione—(BE99199, Ref. 200 M)—An isomeric mixture of ca 60% 5- and 40% 4-(3,4-dichlorophenyl) oxazinedione (3.0 g, 16.3 mmol) was refluxed with dimethyl sulfate (3.0 g, 23.8 mmol) and sodium bicarbonate (2.5 g, 29.8 mmol) in acetone (60 ml) for eight hours. Standard workup resulted in preferential crystallization of the 3-methylated 5-isomer 1.41 g (∿75%), mp 183-4° (dec). Ir ($CDCl_3$) 2990(w), 1790(s), 1730(s), 1640(s), 1600(w), 1560(w), 1450(s), 1320(m), 1180(m), 1120(m), 1090(s), 1020(m), 970(m) cm^{-1}. Pmr (acetone-d_6) δ8.1 (s,1,C_4-H), 7.8 (d or d,1,J_1=8Hz, J_2=2Hz, H_o), 7.55 (m,2,H_o+ H_m), 3.44 (s,3,N-CH_3) PPM. Anal. calcd for $C_{11}H_7CL_2NO_3$: C, 48.55; H, 2.59; N, 5.15; Cl, 26.06. Found: C, 48.60; H, 2.55; N, 5.16; Cl, 25.89.

38. 3-Methyl-4-(3,4-dichlorophenyl)2H-1,3(3H)-oxazine-2,6-dione—(BE99206, Ref. 198 M)—4-(3,4-Dichlorophenyl)oxazinedione (1.0 g, 3.9 mmol) was refluxed with dimethylsulfate (1.4 g 11 mmol), and sodium bicarbonate (1.0 g, 12 mmol)

in acetone (15 ml) for 48 hours. Standard workup gave 525 mg (49%) of 3-methylated product, mp 123-5° (dec). Ir ($CDCl_3$) 3120(w), 1790(s), 1730(vs), 1630(m), 1590(w), 1440(m), 1400(m), 1320(m), 1300(w), 1250(s), 1220(m), 1140(m), 1090(m), 1070(m), 1040(s), 980(m), 820(m) cm^{-1}. Pmr (acetone-d_6) δ7.8 (d,1,J=2Hz, H_o), 7.72 (d,1,J=9Hz, H_m), 7.54 (d of d,1,J_1=9Hz, J_2=2Hz, H_o), 5.80 (s,1,C_5-H), 3.20 (s,3,N-CH_3) PPM. Anal. calcd for $C_{11}H_7Cl_2NO_3$: C, 48.55; H, 2.59; N, 5.15; Cl, 26.06. Found: C, 48.32; H, 2.49; N, 5.09; Cl, 25.96.

39. 4-(p-Fluorophenyl)-2H-1,3(3H)-oxazine-2,6-dione—(BE00601, Ref. 202 M)—p-Fluorophenylmaleic anhydride (1.7 g, 8.9 mmol) was refluxed with trimethylsilyl azide (5 ml, ∿ 36 mmol) and several drops of p-dioxane for two hours. Standard workup gave 850 mg (47%) of isomeric oxazinediones, mp 170-80° (dec). Crystallization from ethyl acetate gave 535 mg of pure 4-isomer, mp 196-8° (dec). Ir (mull) 3120(m), 3170(m), 1780(s), 1725(s), 1705(s), 1625(s), 1580(m), 1300(m), 1220(m), 1160(m), 1080(m), 1030(w), 975(m), 840(m), 820(m), 760(m) cm^{-1}. Pmr (acetone-d_6) δ10.0 (broad, 1, seen only in integration, N-H), 7.84 (d of d, H_o,2,J_{om}=10Hz, J_{oF}=5Hz), 7.28 (t,2,H_m,J_{om}=J_{mF}=10Hz), 5.84 (s,1,C_5-H) PPM. Anal. calcd fo $C_{10}H_6FNO_3$: C, 57.98; H, 2.92; N, 6.76; F, 9.17. Found: C, 58.05; H, 2.91; N, 6.70; F, 9.20.

40. p-Fluorophenyl maleic anhydride—(BG03979, Ref. 203 M)—This compound, previously unreported, was prepared by the method of Hill[2]. p-Fluorophenylsuccinic acid[8] (3.3 g, 15.6 mmol) was refluxed with selenium dioxide (1.9 g, 17 mmol) in acetic anhydride (40 ml) for 24 hours. The precipitated selenium was removed by filtration through a sintered glass funnel and the

filtrate concentrated under reduced pressure yielding a brown solid. The solid was washed with copious volumes of ether and hexane yielding 1.8 g (60%) of tan crystalline solid, mp 112-14°. Ir ($CDCl_3$) 3140(w), 1860(m), 1840(m), 1810(m), 1770(vs), 1620(m), 1600(s), 1505(s), 1415(w), 1310(w), 1300(m), 1290(w), 1225(vs), 1160(s), 1090(m), 1050(m), 1005(w), 830(s), 800(m) cm^{-1}. Pmr (DMSO-d_6) δ8.1 (d of d, 2,H_o,J_{om}=10Hz, J_{oF}=5Hz), 7.55 (s,1,H_α), 7.26 (t,2,H_m, J_{om}=J_{mF}=10Hz) PPM.

41. p-Fluorophenyl succinic acid—(BG03997, Ref. AM 1)—This compound, previously reported[8], was prepared by the organic synthesis procedure[3].

42. 3-Methyl-4(p-fluorophenyl)-2H-1,3(3H)-oxazine-2,6-dione—(BG04001, Ref. 207 M)—4-(p-Fluorophenyl) oxazinedione (1.2 g, 5.8 mmol) was refluxed with dimethyl sulfate (1.1 g, 8.7 mmol) and sodium bicarbonate (1.2 g, 14 mmol) in acetone (30 ml) for 18 hours. Standard workup gave a yellow semisolid which was taken up in the minimum of ethyl acetate (∿ 5 ml). Addition of n-hexane to the solution to a point of permanent turbidity and cooling resulted in precipitation of 1.09 g (85%) of 3-methylated product, white crystals, mp 108-10°. Ir ($CDCl_3$) 3120(w), 1785(s), 1720(s), 1625(s), 1605(m), 1510(s), 1430(s), 1380(s), 1240(s), 1220(m), 1205(m), 1155(s), 1090(m), 1065(m), 1010(m), 1005(m), 960(m), 830(s), 805(s) cm^{-1}. Pmr ($CDCl_3$) δ7.38 (4, AB pattern, aromatics), 5.68 (s,1,C_5-H), 3.28 (s,3,N-CH_3) PPM. Anal. calcd for $C_{11}H_8FNO_3$: C, 59.73; H, 3.65; N, 6.33; F, 8.59. Found: C, 59.64; H, 3.68; N, 6.17; F, 8.55.

43. 3-Methyl-5-(p-fluorophenyl)-2H-1,3(3H)-oxazine-2,6-dione—(BG04010, Ref. 209 M)—An isomeric mixture of 4-(p-fluorophenyl) oxazinedione and the corresponding 5-isomer (∿ 1:1 molar ratio, 3.3 g, 16 mmol) was refluxed with dimethylsulfate (3.0 g, 24 mmol) and sodium bicarbonate (2.7 g, 32 mmol) for 18 hours in acetone (75 ml). Standard workup resulted in preferential crystallization of yellow-white microcrystals (1.02 g, ∿ 66%), mp 155-7° (dec). Ir ($CDCl_3$) 3110(w), 1790(s), 1730(s), 1640(s), 1605(m), 1520(s), 1430(d,m), 1360(m), 1330(s), 1300(m), 1230(s), 1160(s), 1080(s), 1005(m), 980(s), 830(s) cm^{-1}. Pmr (DMSO-d_6) δ8.05 (s,1,C_4-H), 7.55 (d of d with long range splitting, 2,H_o, J_{om}=9Hz, J_{oF}=5.5Hz), 7.10 (t, with long range splitting, H_m, $J_{om}=J_{mF}$=9Hz), 3.40 (s,3,N-CH_3) PPM. Anal. calcd for $C_{11}H_8FNO_3$: C, 59.73; H, 3.65; N, 6.33; F, 8.59. Found: C, 59.69; H, 3.59; N, 6.18; F, 8.65.

44. 2-(3-Chloro-4-methyl phenyl) maleic anhydride—(BG10670, Ref. 210 M)—This compound, previously unreported, was prepared by the procedure of Hill[2] for the preparation of aryl maleic anhydrides mp 98-102°. Ir ($CDCL_3$) 3070(w), 2990(w), 1840(m), 1770(s), 1610(m), 1550(m), 1060(m), 1040(m), 980(m), 810(m) cm^{-1}. Pmr ($CDCl_3$) δ7.95 (d,1,H_o), 7.75 (d of d, 1,H_o),7.40 (d,1,H_m), 7.05 (s,1, olefinic), 2.50 (s,3,AR-CH_3).

45. 2-(3-Chloro-4-methyl phenyl) succinic acid—(BG10689, Ref. Am 2)—This compound, previously unreported, was prepared by the organic synthesis procedure[3] for aryl succinic acids, mp 195-200°. Ir (mull) 1690(s), 1589(w), 1505(m), 1300(m), 1225(m), 1195(m), 1170(m), 1150(w), 1090(w), 1060(w), 1020(w), 1000(w), 920(m), 820(w), 780(w) cm^{-1}. Pmr (DMSO-d_6) δ11.2 (s, broad, 2H, C-OH), 7.2 (m,3, aromatics), 3.9 (d of d,1, J_1=10Hz, J_2=6Hz,

benzylic) 3.0 (d of d, 2, J_1=10Hz, J_2=6Hz, $\underline{CH}_2$-C(=O)-OH), 2.5 (s,3, AR-$\underline{CH}_3$).

46. 4-(3-Chloro-4-methyl phenyl)-2H-1,3(3H)-oxazine-2,6-dione—(BG11382, Ref. 211 M)—2-(3-Chloro-4-methyl phenyl) maleic anhydride (5.3 g 23.8 mmol) was refluxed with trimethylsilylazide (11 ml, ∿75 mmol) and p-dioxane (0.5 ml) for two hours. The solution was cooled to 0° and 40 ml benzene added. Hydrolysis at 0° with 1.2 g (25 mmol) of absolute ethanol caused precipitation of 2.4 g (42%) of isomeric oxazinedione, mp 170-6° (dec). Crystallization from ethyl acetate gave 1.59 g of pure 4-isomer, mp 184-6° (dec). Ir ($CDCl_3$) 3400(w), 1795(s), 1740(s), 1720(s), 1640(m), 1560(w), 1160(w), 1090(m), 1050(w), 980(m), 805(m) cm^{-1}. Pmr (Acetone-d_6) δ∿10 (broad, 1, seen only in integration, N-$\underline{H}$), 7.85 (d,1,H_o), 7.65 (distorted d of d, 1, H_o), 7.45 (d,1,H_m), 5.95 (s,1,C_5-$\underline{H}$), 2.40 (s,3, AR-$\underline{CH}_3$). Anal. calcd for $C_{11}H_8ClNO_3$: C, 55.60; H, 3.39; N, 5.89; Cl, 14.92. Found: C, 55.47; H, 3.40; N, 5.73; Cl, 14.85.

47. 4-(p-Bromophenyl)-2H-1,3(3H)-oxazine-2,6-dione—(BG12763, Ref. 216 M)—p-Bromophenyl maleic anhydride[1] (3.0 g, 11.86 mmol) was refluxed with trimethylsilylazide (7 ml, ∿ 50 mmol) and p-dioxane (1 ml) for three hours. The solution was cooled to 0° and 45 ml benzene added. Hydrolysis with 600 mg (13 mmol) of absolute ethanol at 0° gave 1.1 g (35%) of crude isomeric oxazinediones mp 185-8° (dec). Crystallization of the crude product from ethyl acetate gave 500 mg of pure 4-isomer, mp 209-11° (dec). Ir (mull) 3220(w), 3160(w), 3100(w), 1800(s), 1710(s), 1630(s), 1595(m), 1500(m), 1400(w), 1305(w), 1270(w), 1110(m), 1085(m), 1070(m), 1005(w), 980(m), 840(m), 805(m), 750(m) cm^{-1}. Pmr (DMSO-d_6) δ∿12 (broad, 1, seen

only in integration, N-H), 7.70 (distorted AB pattern, 4, aromatics), 5.66 (s,1,C_5-H). Anal. calcd for $C_{10}H_6BrNO_3$: C, 44.80; H, 2.26; N, 5.23; Br, 29.81. Found: C, 44.74; H, 2.17; N, 5.18; Br, 29.79.

48. p-Bromophenyl maleic anhydride—(BG12772, Ref. 215 M)—This compound, previously reported[1], was prepared by the procedure of Hill[2] for the synthesis of aryl maleic anhydrides.

49. Preparation of ethyl 4-fluoro-3-carboethoxyamino-2-butenoate—(BG14212, KP-III-287, KP-IV-3)—A mixture of 6.8 g (46 mmole) of ethyl 4-fluoroacetoacetate, 3.6 g (40 mmole) of ethyl carbamate and 15 drops of phosphoryl chloride was heated at 50-55° for 10-15 hours. This reaction was followed by glpc using a 5' x 1/4" column of 20% S.E. 30 absorbed on 80/60 chromosorb W at 150°. The low-boiling materials were removed by Kugel-rohr distillation by heating the reaction mixture up to 50° at 0.10-0.15 mm. (Distillation was very slow. Kugel-rohr distillation was continued for ca. 7 hours.) This yielded 5.8 g (26.5 mmole, 66%) of residual white solid which was almost pure ethyl 4-fluoro-3-(N-carboethoxy)amino-2-butenoate. This solid could be distilled out by Kugel-rohr at 80-90°, 0.10-0.15 mm to give an analytically pure sample, mp 43-46°. Ir (mull) 3240(w), 1745(vs), 1680(s), 1640(vs), 1255(vs), 1210(s), 1160(vs), 1045(s), 1030(vs), and 740(m) cm^{-1}. Nmr ($CDCl_3$) δ10.53 (broad s,1), 5.47 (d of d, 2,J_1=48Hz, J_2=1.5Hz), 5.35 (m,1), 4.14 (q,2,J=7Hz), 4.17 (q,2,J=7Hz) and 1.27 (t,6,J=7Hz).

50. 4-(Fluoromethyl)-2H-1,3(3H)-oxazine-2,6-dione—(BG14221, KP-III-289, 291)—A mixture of 4 g (18 mmole) of ethyl 4-fluoro-3-(N-carboethoxy)amino-2-butenoate and ca. 7 ml of polyphosphoric acid (MC&B) was heated at 70-75°

for 2 hours while being stirred manually. The reaction mixture turned dark-brown. After cooling, it was decomposed with 100 ml of ice-water, then extracted with 50 ml of chloroform. The aqueous layer was separated. The chloroform layer was extracted with 50 ml of distilled water. The aqueous layers were combined and extracted with ethyl acetate (1x100 ml, 4x50 ml). The ethyl acetate layers were combined, dried over sodium sulfate and concentrated to give a residual solid which was recrystallized from etherethyl acetate to afford 0.41 g (2.8 mmole, 15%) of analytically pure 4-fluoromethyl-2H-1,3(3H)-oxazine-2,6-dione, mp 132-133° (dec). Ir (mull) 3250(m), 3125(w), 1790(s), 1740(vs), 1655(s), 1355(m), 1070(m), 1020(m), 980(m) and 750(m) cm^{-1}. Nmr (DMSO-d_6-$CDCl_3$) δ11.64 (broad s, 1), 5.62 (s,1, olefinic H) and 5.11 (d,2,J=46Hz, -CH_2F). Anal. calcd for $C_5H_4FNO_3$: C, 41.39; H, 2.78; N, 9.65; F, 13.09. Found: C, 41.55; H, 2.90; N, 9.62; F, 12.99.

51. 4-(Trifluoromethyl)-2H-1,3(3H)-oxazine-2,6-dione—(BG37895, KP-IV-13, 15, 17

i) Reaction of ethyl 4,4,4-trifluoroacetoacetate with ethyl carbamate

A mixture of 6.6 g (36 mmole) of ethyl 4,4,4-trifluoroacetoacetate, 2.7 g (30 mmole) of ethyl carbamate and 1.5 g (10 mmole) of phosphoryl chloride was heated at 60-65° for 16 hours. Some white gummy material adhered to the wall of the reaction flask. Glpc analysis using a 5' x 1/4" column of 20% S.E. 30 absorbed on 80/60 chromosorb W at 165° showed that almost all the ethyl carbamate had reacted. The reaction mixture was distilled by Kugel-rohr to afford 3.6 g (14.1 mmole, 47%) of almost pure ethyl 4,4,4-trifluoro-3-(N-carboethoxy)-amino-2-butenoate as the distillate and ca.

4 g of crude ethyl 4,4,4-trifluoro-3,3-bis(N-carboethoxy)aminobutenoate as the residue by heating the bath up to 85° at 0.10-0.15 mm. The unreacted starting materials were trapped in the condenser cooled by a dry ice-acetone bath.

Analytically pure ethyl 4,4,4-trifluoro-3-(N-carboethoxy)-amino-2-butenoate and ethyl 4,4,4-trifluoro-3,3-bis(N-carboethoxy)aminobutenoate were obtained by column chromatography using chloroform as an eluent on silica gel.

Ethyl 4,4,4-trifluoro-3-(N-carboethoxy)amino-2-butenoate:
Ir (neat) 3260(w), 1765(vs), 1696(s), 1645(vs), 1505(m), 1360(m), 1290(vs), 1200-1140(vs), 1050(vs), 1020(s) and 745(vs) cm^{-1}. Nmr ($CDCl_3$) δ9.93 (broad s,1), 5.70(s,1), 4.23 (q,2,J=7Hz), 4.20 (q,2,J=7Hz) and 1.30 (t,6,J=7Hz).

Ethyl 4,4,4-trifluoro-3,3-bis(N-carboethoxy)aminobutenoate:
Ir (neat) 3350(m), 1750(vs), 1720(vs), 1520(s), 1260(vs), 1180(vs), 1050(s), and 760(m) cm^{-1}. Nmr ($CDCl_3$) 7.17 (broad s,2), 4.35-4.0 (m,6), 3.45 (s,2) and 1.35-1.15 (m,9).

ii) Cyclization of ethyl 4,4,4-trifluoro-3-(N-carboethoxy)-amino-2-butenoate to 4-trifluoromethyloxazinedione

A mixture of 2.9 g (11.4 mmole) of ethyl 4,4,4-trifluoro-3-(N-carboethoxy)amino-2-butenoate and 5 ml of polyphosphoric acid (MC&B) was heated at 105-110° for 15 hours. The reaction mixture was decomposed with 60 ml of ice-water, then extracted with 30 ml of chloroform. The chloroform layer was separated and extracted once with 30 ml of distilled water. The aqueous layers were combined and extracted with ethyl acetate (3x50 ml). The combined ethyl acetate

layers were dried over sodium sulfate and concentrated to yield a residual solid. This solid was triturated with chloroform to afford 0.27 g (1.5 mmole, 13%) of analytically pure 4-trifluoromethyl-2H-1,3(3H)-oxazine-2,6-dione, mp 122-124° (dec). Ir (mull) 3180(m), 3030(w), 1815(s), 1760 (vs), 1680(m), 1540(m), 1325(s), 1270(s), 1210(s) and 1155(s) cm^{-1}. Nmr (DMSO-d_6-$CDCl_3$) δ9.6 (broad s,1) and 5.98 (s,1). Anal. calcd for $C_5H_2NF_3O_3$: C, 33.17; H, 1.11; N, 7.74; F, 31.48. Found: C, 32.97; H, 1.19; N, 7.79; F, 31.64.

52. 4-(p-Tolyl)-2H-1,3(3H)-oxazine-2,6-dione—(BG38043, Ref. 220 M— p-Tolyl maleic anhydride[1] (9.4 g 50 mmol) was refluxed with trimethylsilyl azide (14 ml, ∿0.1 mol) for two hours, with 1 ml of p-dioxane. The solution was cooled to room temperature, 70 ml benzene added, then cooled to 0°. Hydrolysis at 0° with 2.5 g (55 mmol) of absolute ethanol caused precipitation of 4.5 g (44%) of crude oxazinedione mp 193-6° (dec). Crystallization from ethyl acetate gave 3.9 g of pure 4-isomer, white silkin needles, mp 200-201° (dec). Ir (mull) 3240(m), 3160(m), 3110(m), 1810(s), 1710(s), 1625(s), 1510(m), 1280(w), 1260(w), 1185(m), 1110(m), 1080(m), 1030(w), 980(s), 840(m), 800(s), 740(s) cm^{-1}. Pmr (DMSO-d_6) δ∿10 (broad, 1, seen only in integration N-H), 7.5 (AB pattern, 4, aromatics), 5.90 (s,1,C_5-H), 2.4 (s,3,AR-CH_3). Anal. calcd for $C_{11}H_9NO_3$: C, 65.02; H, 4.46; N, 6.89. Found: C, 64.50; H, 4.46; N, 6.60.

53. 3-Methyl-4-(p-tolyl)-2H-1,3(3H)-oxazine-2,6-dione—(BG41559, Ref. 223 M) 4-(p-Tolyl)-oxazinedione (2.4 g, 12 mmol) was refluxed with dimethyl sulfate (3.0 g, 24 mmol) and sodium bicarbonate (2.5 g, 31 mmol) in acetone (50 ml) for 20 hours. The inorganic solids were removed by filtration and the filtrate concentrated under reduced pressure. The semisolid residue was taken

up in 5 ml hot ethyl acetate, filtered hot and cooled to 0°. Hexane was added dropwise to a point of turbidity. Further cooling gave 1.72 g of white crystalline precipitate (66%), mp 99-100° (dec). Ir ($CDCl_3$) 3120(w), 2960(m), 1780(vs), 1720(vs), 1620(s), 1510(m), 1470(s), 1430(s), 1390(m), 1320(s), 1240(m), 1200(m), 1180(m), 1080(m), 1060(m), 1010(m), 1005(m), 960(m), 840(s), 800(s) cm^{-1}. Pmr ($CDCl_3$) δ7.3 (4, distorted AB pattern, aromatics), 5.5 (s,1,C_5-H), 3.2 (s,3,N-CH_3), 2.4 (s,3,Ph-CH_3). Anal. calcd for $C_{12}H_{11}NO_3$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.41; H, 5.20; N, 6.33.

References

1. M. Artico and Y. Nacci, Farmaco Ed. Sci. 20, 523 (1965), Chem. Abstr. 63, 8274e (1965).

2. R.K. Hill, J. Org. Chem., 26, 4745 (1961).

3. C.F.H. Allen and H.B. Johnson, Org. Syn. Coll. Vol. 4, 804 (1963).

4. H.L. Coonradt, H.D. Hartough and H.D. Harris, J. Amer. Chem. Soc. 74, 163 (1952).

5. H.D. Scharf and A. Wittig, Chem. Ber., 106, 1707 (1973).

6. A. Genge, J. India Chem. Soc. 34, 915 (1957).

7. W. Zerweck and K. Schutz, German Patent 870, 106 (to Casella Farbwerke Mainkur, A.G.) Chem. Abstr., 50, 16864c (1956).

8. U. Tadeusz, and J. Lange, Rocz. Chem., 33, 197 (1959). Chem. Abstr., 53, 10749d (1959).

Appendix

ATTEMPTS TO PREPARE FLUORINATED 2H-1,3-OXAZINE-2,6-DIONES

With the exception of four compounds listed in the prepared structures section [4-p-fluorophenyl-, 4-fluoromethyl-, 4-trifluoromethyl-, and 5-p-fluorophenyl-1,3-oxazine-2,6-diones] and their derived N-methylated derivatives, all other attempts to prepare new fluorinated oxazinediones met with little or no success. This appendix details preparative attempts to five structures: 5-methyl-4-trifluoromethyl-, 4-methyl-5-trifluoromethyl-, 4-trifluoromethyl-5-carboethoxymethyl-, 4-methyl-5-fluoro-, and 5-fluoro-1,3-oxazine-2,6-dione.

A. Starting Materials—

1. Ethyl 4,4,4-trifluoro-2-methylacetoacetate:—A mixture of 20 mmol of ethyl trifluoroacetate and 25 ml of dry ether was treated with 20 mmol of ether-washed sodium hydride. To the stirred suspension, 25 mmol of ethyl propionate was added dropwise, then the mixture was heated at reflux for six hours. The mixture was acidified with conc. HCl, then extracted with 5x20 ml of ether. Distillation afforded 40% of pure ketoester; ir (neat liq) 3400, 3000, 1780, 1750, 1680, 1200 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 1.25 (t,3), 1.5 (d,3), 3.9 (q,2), 4.2 (m), and 12.7 (broad) ppm. With other bases, the yields were lower; Na sand, 30%; tBuOK, 10%; EtONa, 10%.

2. Diethyl trifluoroacetosuccinate:—Following the procedure of P. Brown, Tetrahedron, 10, 164 (1960), $EtO_2CCH_2CH(CO_2Et)COCF_3$ was prepared from

CF_3CO_2H and $EtO_2CCH_2CH_2CO_2Et$ with sodium sand in ether.

3. Attempts to prepare Ethyl 2-trifluoromethylacetoacetate:—

a) Direct trifluoromethylation—Into a dry 100 ml Carius tube were charged 20 mmol of ethyl acetoacetate and 20 mmol of potassium t-butoxide. After hydrogen evolution had ceased, the tube was cooled to -78° and 25 mmol of trifluoromethyl iodide condensed therein. The tube was sealed, then heated at 60° for 1.5 hr, at which time the contents were dark brown. It was then left standing at 50° for 1- hr, then opened and acidified with HCl, then extracted with ether. No C-F absorption was evident in the ir spectrum of the ether extract. The major portion (ca 85%) of the trifluoromethyl iodide was recovered unchanged. Similar lack of reactivity was observed with other bases: sodium hydride, mixture heated at 90° for three days; sodium ethoxide, mixture heated at 80° for two days.

b) Trifluoromethylation of thallous ethyl acetoacetate. A mixture of 20 mmol of the thallium salt of ethyl acetoacetate and 20 mmol of trifluoromethyl iodide was sealed in a 100 ml tube as described in (a). After heating for 12 hr at 110°, the light brown reaction mixture was opened, and 2.0 g (51%) of trifluoromethyl iodide recovered. Glpc analysis (silicone oil, 70°) showed a small peak in the retention time zone expected for product, but the ir spectrum of the reaction mixture indicated no C-F bonds.

4. Attempt to prepare Ethyl 2-fluoroacetoacetate:—Following the procedure of E. D. Bergmann, S. Cohen, and I. Shanak, J. Chem. Soc., 3278 (1959) which had been successfully used to prepare ethyl 4-fluoroacetoacetate,

a stirred mixture of 12 g (0.5 mol) of magnesium turnings, 1 g of mercuric chloride, and 53 g (0.5 mol) of ethylfluoroacetate was treated with a 15 ml portion of 61 g (0.5 mol) of ethyl chloroacetate in 100 ml of ether. This was heated at reflux until an exothermic reaction set in, then the balance of the chloroacetate solution was added at such a rate that the mixture maintained gentle reflux without external heating. At the conclusion of the addition, the mixture was heated at reflux for 30 min, cooled, and decomposed with 200 g of ice and 28 ml of conc. H_2SO_4. The ethereal layer was separated and combined with a 1 x 200 ml ether extract of the aqueous phase. After drying (Na_2SO_4), concentration under reduced pressure, and distillation there was obtained 13 g (17% yield) of ethyl 4-fluoroacetoacetate, bp 85-87° (20 mm). Analysis of this fraction, as well as forerun and postruns, by glpc (silicone oil) revealed no presence of ethyl 2-fluoroacetoacetate.

B. Fluorinate Oxaziniones

1. Reaction of Ethyl 4,4,4-trifluoro-2-methylacetoacetate with Ethyl Carbamate:—general procedure of S. S. Washburne and K. K. Park, Tetrahedron Lett., 243 (1976).

a) A mixture of 20 mmol of ethyl 4,4,4-trifluoro-2-methylacetoacetate, 20 mmol of ethyl carbamate, 1.4 g of phosphorus pentoxide, and 10 ml of phosphorus oxychloride were stirred for 10 min in a flame-dried 100 ml flask, then heated at 85° for 3.5 hr. The light brown reaction mixture slowly evolved HCl gas. After cooling to room temperature, $POCl_3$ was stripped from the reaction mixture, 50 ml of water cautiously added, and the mixture brought to neutrality by addition of 10% sodium hydroxide

solution. The mixture was extracted with 3 x 100 ml of benzene and 4 x 75 ml of ethyl acetate, and the combined extracts dried (Na_2SO_4), concentrated to 25 ml and stored at -20°. After two hours a small crop of white crystal separated. These were collected and identified as ethyl allophanate ($EtO_2CNHCONH_2$). Further concentration and cooling afforded a small yield of recovered ethyl carbamate, and complete evaporation of the organic layers afforded unreacted starting ketoester.

b) Under similar conditions 20 mmol quantities of ketoester and ethyl carbamate were heated with 15 drops of $POCl_3$ for 2 hr at 55°, then for 12 hr at 85°. Glpc monitoring showed slow growth of a new peak of longer retention time than starting materials, but workup as in (a) afforded only ethyl allophanate.

c) A mixture of 20 mmol of ketoester, 25 mmoles of ethyl carbamate, 5 drops of $POCl_3$ and 20 ml of benzene was placed in a Soxhlet extractor, the thimble of which was charged with freshly activated Linde Molecular Sieve 3A. After three days of reflux, during which time the reaction was monitored by tlc (mobile phase: EtOAc), no product formation was observed.

d) A mixture of 20 mmol each of ketoester and ethyl carbamate, and 10 mmol of $POCl_3$ were heated at 55° for 40 hr. No product formation was apparent by glpc. A further 10 mmol of $POCl_3$ was added and the mixture heated at 60° for 48hr. A small quantity of white crystals identified as ethyl allophanate adhered to the sides of the reaction flask.

e) A mixture of 15 mmol of ketoester and 20 mmol of benzyl carbamate were heated at 90° for 1.5 hr. After addition of 0.3 ml of

$POCl_3$, heating was continued for 48 hr. Glpc analysis indicated the presence of ketoester (ca 80% recovery) and benzyl alcohol.

2. Reaction of Diethyl trifluoroacetosuccinate with Ethyl Carbamate:—Procedure as in 1 above.

a) A mixture of 10 mmol each of diethyl trifluoroacetosuccinate and ethyl carbamate were heated with 10 ml of $POCl_3$ for 14 hr at 85°. Phosphorus oxychloride was stripped from the red reaction mixture and 100 ml of chloroform added. This solution was extracted with 5 x 100 ml of water and the aqueous extracts back extracted with 2 x 350 ml of ethylacetate. Evaporation of the ethyl acetate layer afforded a viscous oil which showed at least five new spots upon tlc examination, and was not worked up further.

b) Similar quantities of reactants as in (a) were heated at 70° for 24 hr. After removal of $POCl_3$ (Kugelrohr), 50 ml of benzene was added. The solution was extracted with 3 x 50 ml of water, and the aqueous extracts neutralized with NaOH solution, then back extracted with 3 x 15 ml of ethyl acetate. The dried ethyl acetate layer was concentrated to 10 ml by rotary evaporation. A deposit of white crystals was identified as ethyl allophanate. The filtrate was concentrated to 5 ml, then refrigerated (-20°) for 48 hr. No further crystallization occured. Tlc analysis of this filtrate showed at least four product spots.

3. Attempted Isolation of 5-Fluoro-1,3-oxazine-2,6-dione from reaction mixture of fluoromaleic anhydride and trimethylsilyl azide:—
As previously published (J. D. warren, J. H. MacMillan, and S. S. Washburne,

J. Org. Chem., 40, 743 [1975]) reaction of fluoromaleic anhydride with trimethylsilyl azide gives a ten percent yield of 4-fluoro-1,3-oxazine-2,6-dione. Subsequent to our observation of varying proportions of 5-aryl-oxazinediones as a minor product from reaction of arylmaleic anhydrides with trimethylsilyl azide, we succeeded in isolating a small quantity of 5-methyl-1,3-oxazine-2,6-dione from the mother liquors of the reaction of citraconic anhydride with trimethylsilyl azide (reported in JOC 40, 743 (1975) to give only the 4-isomer), by the technique of preparative tlc with monitoring of the characteristic carbonyl ir region of oxazine-diones. That reaction of trimethylsilyl azide with citraconic anhydride gives both isomers has been confirmed (J. Farkaš, O. Fliegerová, and J. J. Škoda, Collect. Czech. Chem. Commun., 41, 2059 [1976]). By similar preparative tlc we examined the by now two year old reaction micture of fluoromaleic anhydride with trimethylsilyl azide, but no products with oxazinedione ir spectra were observed. This project was terminated by cessation of support in June 1976.

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